# INFLUENCE OF FATIGUE ON THE DETERMINANTS OF ENDURANCE EXERCISE PERFORMANCE

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**Explanatory author's declaration**

All publications included in the thesis were written by the author under the previous (maiden) name of Ida Clark.

**Abstract**

Both psychological and physiological factors contribute to exercise performance at different exercise intensities. Measuring the characteristic physiological responses of an athlete at different exercise intensities makes it possible to predict the tolerance for exercise at a given work rate using the so-called power-duration relationship. While estimating athletic performance in this way is widely practiced, it is possible that the physiological responses typically measured at a given work rate are altered by factors relating to fatigue during long duration events. The purpose of this thesis was to investigate the plasticity of the power-duration relationship in the face of psychological stress and prolonged fatiguing exercise. Firstly, the thesis showed that severe-intensity time trial performance was not different after a prolonged cognitive function task compared to control, in either untrained men or in competitive athletes. Secondly, the thesis investigated the effects of prolonged, fatiguing endurance exercise on the power-duration relationship. Initially, the power asymptote of the hyperbolic power-time relationship critical power (CP) or end test power and the curvature constant of this relationship W' or work done above end test power were not different and highly correlated when estimated from two different 3-min all-out exercise tests (3MT) preceded by 2 h of heavy-intensity exercise. After 2 h of heavy-intensity exercise both EP and WEP were lower compared to no prior exercise (control). Importantly, critical power and W′ established from three separate severe-intensity predication trials conducted immediately following 2 h of heavy-intensity exercise did not differ from F-EP and F-WEP established from a 3MT. F-EP and F-CP as well as F-W′ and F-WEP was ~11% and ~20% lower than C-EP and C-WEP, respectively. Furthermore, C-EP

estimated from a 3MT was not different when established after 40 min, 80 min and 2 h of prior heavy-intensity exercise consuming carbohydrates. However, EP estimated after 2 h of heavy-intensity exercise without carbohydrate consumption was lower than all. C-WEP was higher compared to WEP estimated after 80 min of prior heavy-intensity exercise, 2 h of heavy-intensity exercise with and without carbohydrate consumption but was not different compared to estimates established after 40 min of prior heavy-intensity exercise. Firstly, this thesis has demonstrated that time trial performance is not affected by the psychological stress induced by prolonged cognitive tasks in trained athletes. Secondly, it has been demonstrated that prolonged heavy-intensity exercise alters both CP and W′. Thirdly, the results showed that the 3MT is a reliable and valid test to estimate CP and W′ in a fatigued state and that the decrease in CP, but not W′, after prolonged fatiguing exercise can be mitigated by the consumption of carbohydrates.

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**Symbols and abbreviations**

| | |
|---|---|
| [] | concentration |
| Δ | delta (change) |
| > | above |
| < | below |
| 3MT | 3-min all-out exercise test |
| ACC | anterior cingulate cortex |
| ADP | adenosine diphosphate |
| ATP | adenosine triphosphate |
| $Ca^{2+}$ | calcium |
| C-EP | end test power estimated with no prior exercise |
| CNS | central nervous system |
| $CO_2$ | carbon dioxide |
| CP | critical power |
| CV% | coefficient of variation |
| C-WEP | work above end test power estimated with no prior exercise |
| EP | end test power |
| F-3MT | fatigued 3-min all-out test |
| F-CP | fatigued critical power |
| F-EP | fatigued end test power |
| F-W' | fatigued W' |
| F-WEP | fatigued work done above end test power |
| GET | gas exchange threshold |
| h | hour |
| $H^+$ | hydrogen ion |
| HR | Heart rate |
| $K^+$ | potassium |
| kJ | kilojoule |

| | |
|---|---|
| L | liter |
| LT | lactate threshold |
| min | minute |
| ml | milliliter |
| $Na^+$ | sodium |
| $O_2$ | oxygen |
| PCr | phosphocreatine |
| PFK | phosphofructokinase |
| $P_i$ | inorganic phosphate |
| rpm | revolutions per minute (cadence) |
| s | seconds |
| SR | sarcoplasmic reticulum |
| $T_{lim}$ | time to exhaustion |
| $\dot{V}CO_2$ | carbon dioxide output |
| $\dot{V}E$ | ventilator equivalent |
| $\dot{V}O_2$ | oxygen uptake |
| $\dot{V}O_{2max}$ | maximum oxygen uptake |
| $\dot{V}O_{2peak}$ | peak oxygen uptake |
| W' | curvature constant of the power-duration relationship |
| W | Watt |
| WEP | work done above end-test power |

## Publications

**Clark IE,** Vanhatalo A, Bailey SJ, Wylie LJ, Kirby BS, Wilkins BW, Jones AM (2018). Effects of two hours of heavy-intensity exercise on the power-duration relationship. Med Sci Sports Exerc. 50(8):1658–1668.

**Clark IE,** Goulding RP, DiMenna FJ, Bailey SJ, Jones MI, Fulford J, McDonagh STJ, Jones AM, Vanhatalo A (2019). Time-trial performance is not impaired in either competitive athletes or untrained individuals following a prolonged cognitive task. *Eur J Appl Physiol*. 119(1):149-161.

**Clark IE,** Vanhatalo A, Thompson C, Wylie LJ, Bailey SJ, Kirby BS, Wilkins BW, Jones AM. Changes in the power-duration relationship following prolonged exercise: estimation using conventional and all-out test protocols and relationship to muscle glycogen. *Am J Physiol*, 317(1): R59-R67.

**Clark IE,** Vanhatalo A, Thompson C, Joseph C, Backwell JR, Wylie LJ, Ten R, Bailey SJ, Wilkins BW, Kirby BS, Jones AM (2019). Dynamics of the power-duration relationship during prolonged endurance exercise and influence of carbohydrate ingestion. *J Appl Physiol*. (Ahead of print).

**Presentations**

**Clark, I.E.,** Jones, A.M., Bailey, S.J., Kirby, B.S., Wilkins, B.W., Wylie, L.J., Vanhatalo, A. (2017). Effects of Prolonged, fatiguing exercise on critical power: reliability and physiological characterisation. *American College of Sports Medicine Annual Symposium*. Denver, CO.

**Clark, I.E.,** Goulding, R., Bailey, S.J., Jones, A.M., Fulford, J., Mcdonagh, S.T.J., Jones, M.I., Vanhatalo, A. (2016). Mental fatigue does not impair exercise performance in trained or untrained individuals. *European College of Sports Science Annual Congress.* Vienna, Austria.

## *Dedication*

I dedicate this thesis to my Husband, Mom, Sister and Brother. I hope it makes you proud.

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## Chapter 1: Introduction

Athletes have been trying to break world records since the ancient Olympic Games in Olympia, Greece 776 BC. Every year world records are broken as athletes and coaches work to outperform their counterparts. Scientists have been trying to understand the physiology of the human body for centuries in order to better predict and extend human performance. The better we become at understanding the human body, the more effectively we can train athletes and predict their performance.

In 1925 English physiologist A. V. Hill set out to understand the limit of human performance. He built a curve by plotting record performance times in various sports against the distance covered for each record. Hill found that faster speeds were associated with shorter distance events while longer events were associated with slower speeds. Fascinatingly, Hill noticed that the curve “leveled off” at around 12 min representing a hyperbola. This holds true today when plotting the best human performances. Later, in 1965, Monod and Scherrer investigated the maximal work capacity for small muscle mass exercise. They noted that the hyperbolic relationship between the time to exhaustion and power output could be transformed into a linear relationship. The slope of this relationship was termed Critical Power (CP) and the y-intercept termed the “muscles energy reserve” (today known as W'). The CP was theorized as a power output that could be sustained ‘for a very long time without fatigue’ (Monod and Scherrer 1965).

The power asymptote of the hyperbolic relationship is termed CP and the curvature constant is termed W'. These parameters of the hyperbolic power-time relationship are

strong predictors of endurance exercise performance (Jones et al. 2010; Vanhatalo et al. 2011a). CP separates power outputs for which exercise tolerance can be sustained for long periods of time (>30min) from those that are predictably limited. The tolerance for exercise performed above CP is limited by the power output sustained above CP and the size of W' (and its constituents described further in section 2.2), while the tolerance for exercise performed below CP is likely limited by muscle glycogen depletion, central fatigue, muscle damage and dehydration (Burnley et al. 2018; Gonzàlez-Alonso et al. 1997; Poole et al. 2016; St Clair Gibson et al. 2001). During endurance events the submaximal power output is limited to the athlete's CP, i.e., the greater the CP, the faster the pace the athlete can sustain during the event. Recently, Jones and Vanhatalo (2017) analyzed the critical speed (CS; the running equivalent to CP) and D' (the running equivalent to W') of 12 elite marathon runners' personal best times over several distances and concluded that the athletes were running marathon distances at a submaximal speed around 96% of their CS. However, the effect of prolonged, fatiguing exercise on estimates of CP and/or W' is unknown. Subtle changes in the parameters of the power-duration relationship incurred by prolonged exercise may limit the predictive capabilities of this relationship. Theoretically, the lowering of CP during an endurance event (such that by sustaining the same race pace, there comes a point at which the athlete begins to exercise above CP) likely results in the development of premature fatigue thus altering the anticipated completion time. Understanding how CP behaves during endurance events is important for accurate performance predictions as well as optimization of pacing strategies. Therefore, the overarching aim of this

thesis has been to explore how CP and W' change over 2 h of exercise and the underlying mechanisms associated with potential changes.

To date we know there are many factors that limits exercise performance such as physiological and psychobiological factors. There is controversy as to how psychological factors affect exercise performance and therefore part of this thesis was also conducted to investigate the role psychological factors play on severe-intensity exercise performance.

# Chapter 2: Literature Review

## 2.1 Physiological responses to exercise within distinct exercise intensity domains

To understand the metabolic responses to exercise we can use either $^{31}$phosphorous magnetic resonance spectroscopy, muscle biopsies or gas analysis. Gas analysis is the most cost effective as well as a non-invasive way to estimate the muscle metabolic rate during exercise. Almost 100 years ago Hill et al. (1924) observed that the supply of oxygen was either steady or non-steady when exercising at different intensities. Later on, at the end of the 20$^{th}$ century researchers were able to distinguish between different intensity domains by the use of oxygen uptake ($\dot{V}O_2$) kinetics (Jones and Poole 2005; Poole et al. 1988; Whipp et al. 2005; Whipp and Wasserman 1972). This is of high importance as % maximum oxygen uptake ($\dot{V}O_{2max}$) has been widely used to define exercise intensity. Assigning exercise intensity in this way does not account for the possible inter-individual differences in the physiological response to exercise at a given $\%\dot{V}O_{2max}$ (Lansley et al. 2011). For example, blood lactate accumulation is likely to begin at different fractions of $\dot{V}O_{2max}$ in the trained compared to the untrained performer. Moreover, lactate threshold (LT) may differ widely between trained individuals with comparable $\dot{V}O_{2max}$ values (Figure 2.1).

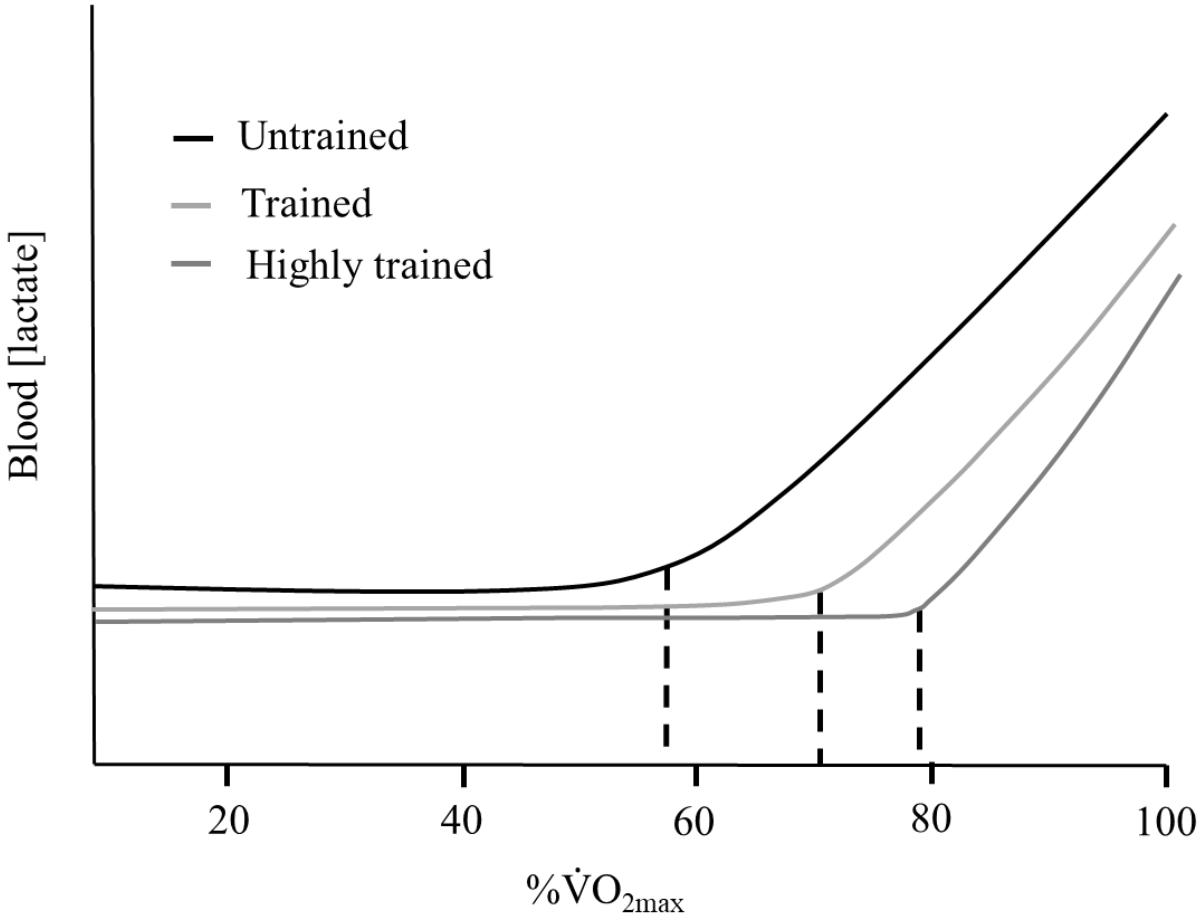


***Figure 2.1.*** *Schematic view of the different relative intensities (%$\dot{V}O_{2max}$) LT may lie at in untrained and trained individuals.*

To eliminate this problem, four different intensity domains have emerged in which predictable physiological responses are obtained; the moderate-, heavy-, severe- and extreme-intensity domains (Hill et al. 2002; Whipp 1994) (Figure 2.2). The extreme-intensity domain includes power outputs at which exhaustion occurs before $\dot{V}O_{2max}$ is attained, typically less than 2 min (Hill et al. 2002). As part of this thesis, the moderate-, heavy- and severe-intensity domain will be reviewed.

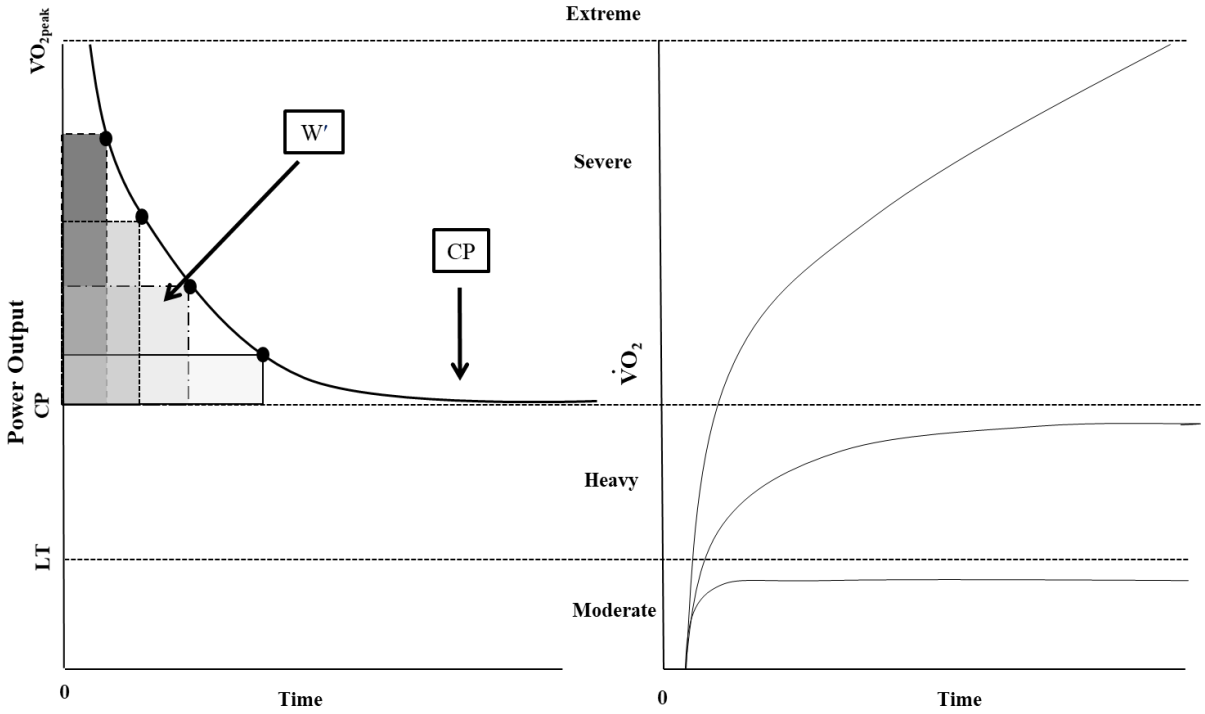


***Figure 2.2.*** *Schematic illustration of the four exercise intensity domains and corresponding oxygen uptake ($\dot{V}O_2$) responses (right panel). A hyperbolic curve is constructed from performance times obtained from four constant work rate cycling trials (black dots; left panel). The dashed line represents CP and the rectangles represent W′. Note the areas of the rectangles are identical and represent a fixed amount of W′ irrespective of work rate within the severe domain (left panel).*

### 2.1.1 Moderate-intensity domain

Exercise within the moderate-intensity domain includes work performed below the LT, or its equivalent, the gas exchange threshold (GET) (Figure 2.2). The LT typically occurs at 50-65% of $\dot{V}O_{2max}$ in healthy individuals and at 70-80% of $\dot{V}O_{2max}$ in well trained individuals (Poole et al. 2016). At the onset of exercise, $\dot{V}O_2$ will increase until a steady-state is attained (typically within 3 min; Burnley and Jones 2007; Gaesser and

Poole 1996; Wilkerson et al. 2004). At the onset of exercise, the demand for ATP cannot be met by oxidative phosphorylation alone and an oxygen deficit is generated. ATP must therefore be resynthesized through PCr and glycolytic pathways resulting in increased $H^+$ and lactate production (Jones and Burnley 2009). When $\dot{V}O_2$ reaches a steady-state, ATP demand can be met by oxidative phosphorylation with no significant elevation in blood lactate (Poole et al 1988; Smith and Jones 2001). It should be noted that during long duration or at high work rates within the moderate-intensity domain there is evidence of a dissociation between $\dot{V}O_2$ and blood lactate (Davis and Thompson 1986). Specifically, lactate remain at resting levels while $\dot{V}O_2$ increases slightly, this is possible due to the increased energy expenditure from muscle bioenergetics (substrate utilization) or motor unit recruitment (neuromuscular fatigue) alterations (Burnley and Jones 2018; Gollnick et al. 1974; Lepers et al. 2002). The increase in $\dot{V}O_2$ is however at a much slower rate than during heavy- or severe-intensity exercise (Burnley and Jones et al. 2011; Gaesser and Poole 1996). Highly-trained individuals have a smaller $O_2$ deficit, and therefore, a smaller $\dot{V}O_2$ time constant (the rate at which $\dot{V}O_2$ rises towards a steady-state). The quicker attainment of a $\dot{V}O_2$ steady-state spares the utilization of muscle glycogen and limits the fall of PCr (Baldwin et al. 1974; Burnley and Jones 2007; Jones and Burnley 2009). In the moderate-intensity domain, exercise can be carried out for several hours with the likely fatiguing mechanism being muscle glycogen depletion, muscle damage, central fatigue or increased core temperature (Black et al. 2017; Burnley and Jones 2007; González-Alonso et al. 1997; St Clair Gibson et al. 2001).

### 2.1.2 Heavy-intensity domain

The heavy-intensity domain lies between the LT and the CP (Figure 2.2). Power outputs within the heavy-intensity domain typically range from 60% to 85% $\dot{V}O_{2max}$ with CP occurring at 70-80% $\dot{V}O_{2max}$ in healthy subjects and 80-90% $\dot{V}O_{2max}$ in well-trained individuals (Burnley and Jones 2018; Poole et al. 2016). During exercise within the heavy-intensity domain, PCr and pH are reduced while $P_i$ is elevated but all typically stabilize within 3 min (Jones et al. 2008) (Figure 2.3). This is accompanied by a greater $\dot{V}O_2$ response than that predicted from sub-LT work rates resulting in a $\dot{V}O_2$ slow component. The $\dot{V}O_2$ slow component is a continuous increase in $\dot{V}O_2$ which ultimately leads to $\dot{V}O_{2max}$, and reflects a loss in muscle efficiency. The mechanistic bases for the $\dot{V}O_2$ slow component are likely due to the increased recruitment of the less efficient type II muscle fibers which contribute to force at a larger $O_2$ cost per unit of external work (Jones and Burnley 2009; Jones et al. 2011; Poole et al. 1991; Vanhatalo et al. 2011b). However, $\dot{V}O_2$ and blood [lactate] typically stabilize in around 10-20 min (Black et al. 2017; Burnley and Jones 2007; Gaesser and Poole 1996; Jones and Poole 2005).

The fatigue process within the heavy-intensity domain is multifaceted and exercise intolerance typically occurs within 40 min to 3 h (Burnley and Jones 2007; Burnley and Jones 2018; Coyle et al. 1986). Fatigue development in the heavy-intensity domain appears to be related to the decrease in energy substrate (glycogen), additionally the depletion in muscle glycogen is more rapid within the heavy-intensity domain compared to the moderate-intensity domain (Black et al. 2017; Coyle et al. 1986; Hermansen et al 1967; Jones and Burnley 2009).

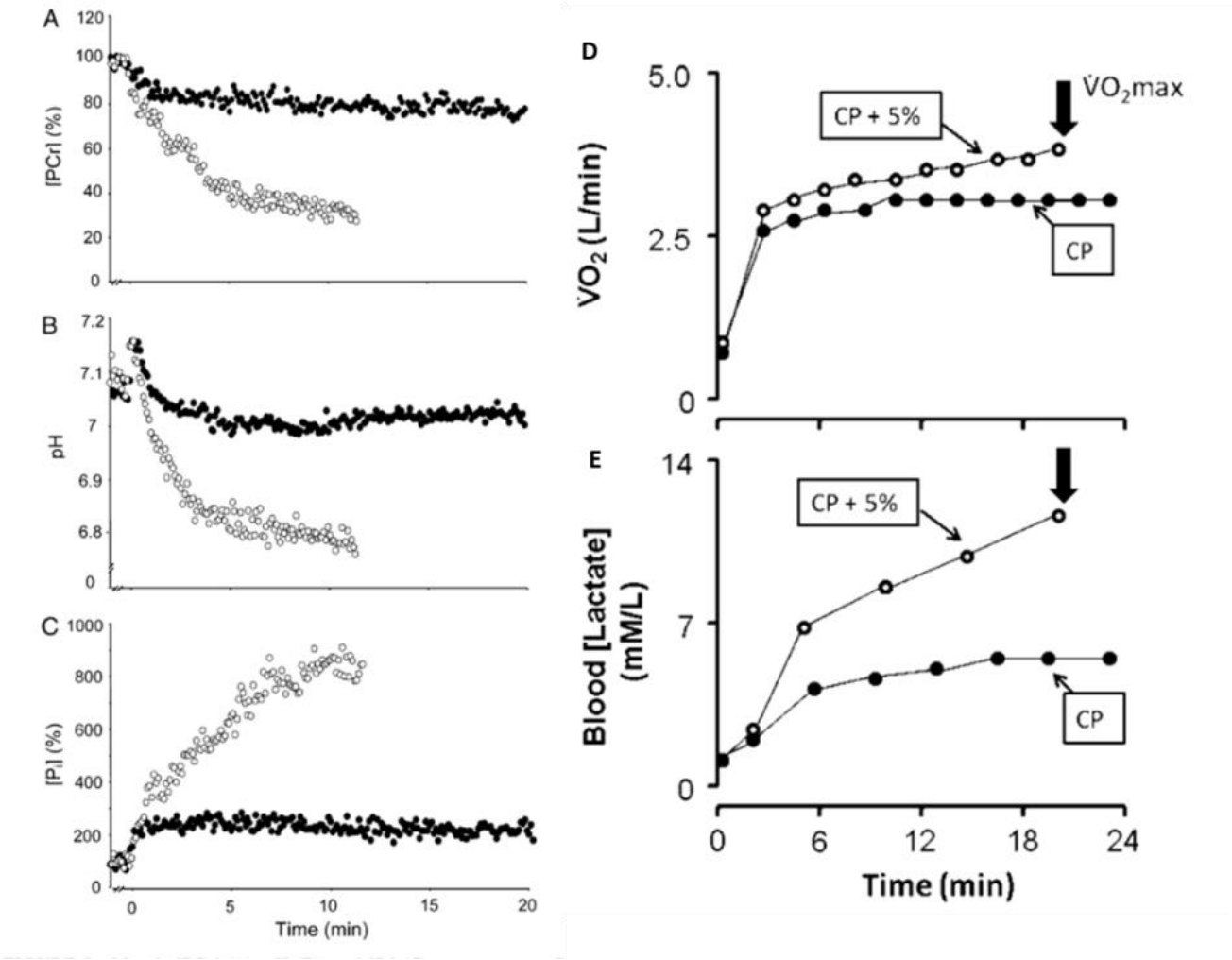


***Figure 2.3****. PCr (A), pH (B) and $P_i$ (C) response to exercise below CP (black) and above CP (white). $\dot{V}O_2$ (D) and blood [lactate] (E) response to exercise intensities at CP (black) and 5% above CP (white). A steady-state (black) and a slow component (white) is achieved in all five variables (A-E). Figures reproduced from Jones et al. 2010.*

### 2.1.3 Severe-intensity domain

The lower boundary of the severe-intensity domain is CP and the upper boundary is the highest power that elicits $\dot{V}O_{2max}$ (Hill et al. 2002; Poole et al. 1988). When exercise is carried out within the severe-intensity domain, $\dot{V}O_2$, muscle metabolic and blood acid-

base responses are unable to stabilize. Specifically, selecting work rates within the severe-intensity domain yields a $\dot{V}O_2$ and a [PCr] ([] denotes concentration) slow component (Black et al. 2017; Jones et al. 2008; Figure 2.3). The $\dot{V}O_2$ slow component will continuously rise for as long as the work rate remains within the severe-intensity domain until $\dot{V}O_{2max}$ is reached and exhaustion ensues (Hill et al. 2002; Poole et al. 1988). The trajectory of the $\dot{V}O_2$ slow component represents the development of fatigue within the severe-intensity domain (Burnley and Jones 2018). Exercising >CP is also known to decrease PCr and pH while increasing $P_i$, blood and muscle [lactate] until critical values are attained at the point of exhaustion (Figure 2.3; Allen et al. 2008; Black et al. 2017; Jones et al. 2008; Vanhatalo et al. 2010). Together these combined influences can impair $Ca^{2+}$ release as well as the sensitivity of the myofilaments to $Ca^{2+}$, ultimately impairing cross bridge cycling (further discussed in chapter 2.4) (Allen et al. 2008; Black et al. 2017; Debold et al 2016; Fitts 1994; Fitts 2008). Exercise within the severe-intensity domain is typically tolerable for <30 min (Burnley and Jones 2018) where exercise duration can be predicted with use of the power-duration relationship (Hill et al. 2002; Monod and Scheerer 1965; Pettitt 2012; Poole et al. 1988).

## 2.2 The power-duration relationship

The power-duration relationship describes the relationship between power output and exercise duration and is used to understand exercise tolerance (Poole et al. 2016).

This relationship can distinguish when energy provision is predominantly provided by substrate-level phosphorylation compared to oxidative phosphorylation as well as estimate individual exercise performances. During long duration exercise, $\dot{V}O_2$, blood

[lactate], PCr turnover, and ATP resynthesis through substrate-level phosphorylation are at a steady-state allowing exercise to be performed for long periods of time. In contrast, when exercise is carried out at high power outputs, a physiological steady-state cannot be maintained, but rather a sustained increase in blood [lactate] and $\dot{V}O_2$ is observed, where $\dot{V}O_{2max}$ is obtained at exhaustion. The CP separates power outputs for which exercise tolerance is predictably limited and is defined as the greatest oxidative metabolic rate that can be sustained without depleting W′ (Poole et al. 2016). W' is a fixed amount of work that is expended at a rate proportional to the power output above CP (Jones et al. 2008; Jones et al. 2010; Monod and Scherrer 1965; Moritani et al. 1981; Figure 2.2). Originally, W' was defined as the anaerobic work capacity (maximal amount of ATP resynthesis via anaerobic metabolism during exhaustive short-duration exercise) (Green 1994; Moritani et al., 1981). However, instead of viewing W' as an expendable anaerobic work capacity it has been posited W' also reflects the accumulation of fatigue metabolites. The greater the power output above CP, the faster the W′ is expended resulting in measurable reductions of intramuscular substrates (PCr and glycogen) and attendant increases of fatigue-related metabolites ($H^+$, ADP, and $P_i$) which accumulate in the blood and/or muscle (Jones et al. 2008; Figure 2.3). Exercising at different power outputs above CP will elicit a consistent ‘critical level’ of fatigue-related metabolites at exhaustion (Vanhatalo et al. 2010). There is a relationship between the depletion of W′ and the development of the $\dot{V}O_2$ slow component during severe-intensity exercise. There is also a positive correlation between the size of W′ and the size of the $\dot{V}O_2$ slow component, which suggests that there is a link between the loss of skeletal muscle efficiency and the development of fatigue (Murgatroyd et al.

2011; Vanhatalo et al. 2011b). The following sections will summarize how CP and W′ are mathematically derived and later, how fatiguing exercise alters these parameters.

The parameters of the power-duration relationship, CP and W', can be used to predict performance in various types of events/sports. Work to date validates these parameters in the prediction of relatively short-duration events in which exercise intensity exceeds CP (i.e. events lasting 2 – 30 min in duration) (Jones et al. 2010; Jones and Vanhatalo 2017; Vanhatalo et al. 2011a). However, a recent review article by Jones and Vanhatalo (2017) estimated that elite distance runners run at 96% of CS during a marathon, and therefore it is possible that the CP concept is useful for the prediction of a race pace during longer duration events as well. This knowledge is valuable when creating race strategies (i.e. the knowledge before the race of what an athlete's best performance times would be over a given distance is of great advantage). However, it remains unclear if the parameters of the power-duration relationship are affected by prolonged endurance activity, which would alter performance predictions. This needs further investigation.

### 2.2.1 The conventional two-parameter critical model

The power-duration relationship describes exercise tolerance and is hyperbolic when plotting power or speed against time. The hyperbolic curve defines the limit of tolerance for severe-intensity exercise. Conventionally, the curve is created by an individual exercising to exhaustion on 3-5 separate occasions at different severe-intensity domain constant power outputs (Hill 1993; see Figure 2.2). The power outputs are selected to elicit exhaustion between 2 and 15 min in duration, after which the time to the limit of

tolerance is recorded. The power-asymptote of the curve is termed CP (measured in Watts) and the curvature constant is named W' (measured in joules) (see Figure 2.2).

The two-parameter model entails three mathematically equivalent equations; one non-linear (hyperbolic model) and two linear (work-time and inverse of time model). The non-linear model is expressed as:

$$T_{lim} = W' / (P - CP) \quad [a]$$

where P is power expressed in Watts, W' in joules, $T_{lim}$ is time to exhaustion (seconds) and CP is expressed in Watts.

The two linear models are the work-time and inverse of time models. In the work-time model the slope is CP and the y-intercept is W':

$$W = (CP \cdot T_{lim}) + W' \quad [b]$$

where W (total work) is in joules, CP is in Watts, $T_{lim}$ is time to exhaustion (seconds) and W' is in joules (see Figure. 2.4A). In the inverse time model CP is the y-intercept and W' is the slope (Whipp et al. 1982)(Figure 2.4B):

$$P = (W' / T_{lim}) + CP \quad [c]$$

With the help of the power-duration relationship, any power output on the hyperbolic curve can be selected resulting in an estimated $T_{lim}$ at that power output.

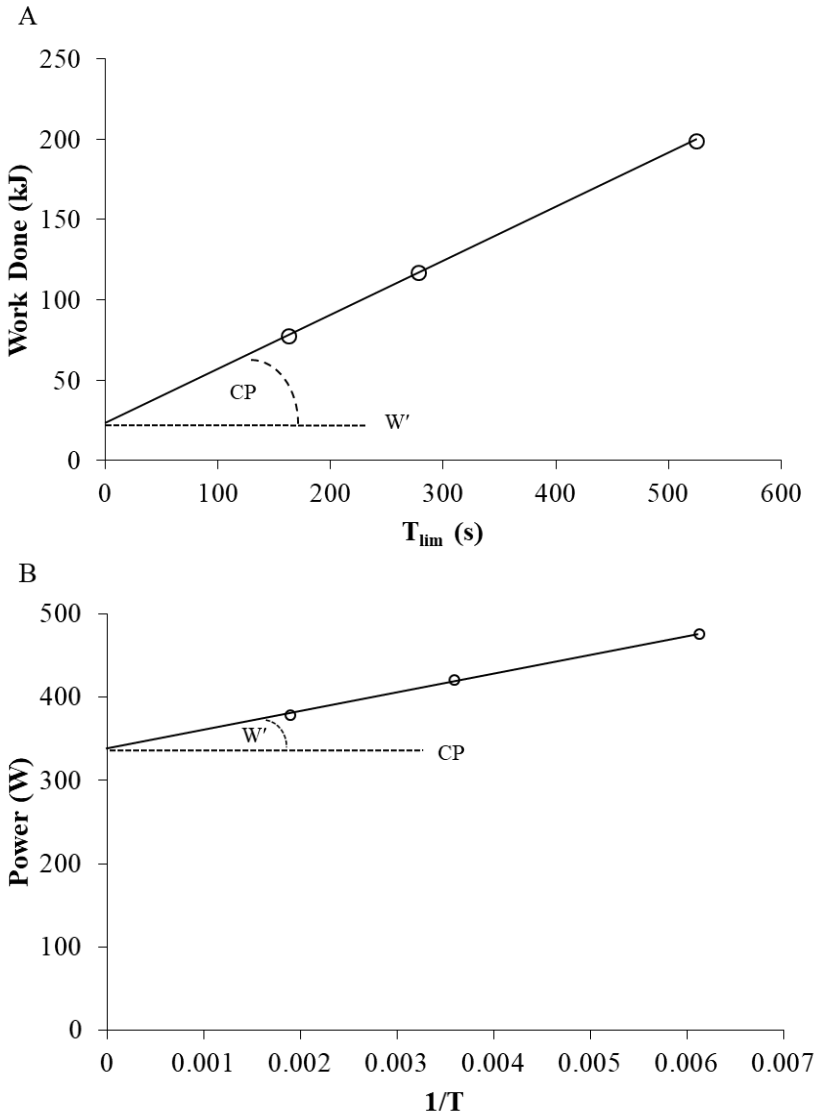


***Figure 2.4.*** *Illustration of the two-parameter hyperbolic work-time (panel A) and 1/time models (panel B). In the in the work-time model the intercept is W' and the slope is CP, yet in the inverse time model the intercept is CP and the slope is W'.*

When the tests are performed during similar conditions the test-retest reliability for CP has a coefficient of variation (CV%) that lies between 3-6% and a correlation coefficient

between 0.92-0.96 while W′ is more variable and can range between 8-17% and correlation coefficient between 0.64-0.87 (Black et al. 2016; Gaesser and Wilson 1988; Hill and Smith 1994; Nebelsick-Gullett et al. 1988; Smith and Hill 1993). Therefore it has been proposed that a standard error of less than 5% for CP and less than 10% for W′ is the acceptable level of accuracy when using the different mathematical models (Hill and Smith 1994).

In addition to the 2-parameter models the 3-parameter model can also be used to estimate CP and W'. The 2-parameter models assumes that it is possible to utilize power outputs which exceed the peak power output, the 3-paremeter model addresses this by taking peak power output into account (Gaesser et al. 1995; Morton 1986):

$$P = (W' / (T_{lim} – (W' / (CP – P_{max}))) + CP \quad [d]$$

Where $P_{max}$ is the highest power output attainable during a maximal effort (from a rested state). Therefore the 3-parameter model is great to use for short durations (0-120s) compared to the 2-parameter models, which would be outside the applicable range of the power-duration relationship. However, it has a tendency of over estimating W' and underestimating CP (Bull et al. 2000) and it has not been validated within the literature for estimating CP. Due to the 3-parameter model not being as precise as the 2-parameter model to predict durations which lays in (best at the shorter durations) the applicable range of the power-duration relationship, the extensive use of the 2-parameter models, and the close agreement of CP and W' estimated using the 2-parameter models, this thesis will be using the 2-parameter models to estimate CP and W'.

### 2.2.2 The 3-min all-out exercise test

In addition to the conventional model, it has been established that the 3-minute all-out exercise test (3MT) is also appropriate for estimating CP and W' (Burnley et al. 2006b). It should be acknowledged that the 3MT is used in a wide variety of exercise modalities such as cycling (Burnley et al. 2006b), rowing (Cheng et al. 2012), running (Pettitt et al. 2012) and swimming (Tsai and Thomas 2016) to estimate CP (CS) and W' (D'). Unlike the conventional model, the 3MT requires a preliminary ramp incremental $\dot{V}O_{2max}$ test and two trials (where the first one acts as a familiarization visit) in which an individual performs an all-out effort. During the test the individual performs a maximal effort for 3 minutes; obviously the starting speed is very high but shortly begins to decrease until it reaches a plateau. The plateau (last 30 s of the test) is equal to CP and the work done above CP (initial 150 s of the test) is W'. In theory, if a subject cycles all-out for a duration of 3 min and it takes 150 s to wholly deplete W', and since W' cannot be replenished until power falls below CP, the remaining power for the last 30 s of the test would be equal to CP (Burnley et al. 2006b; Vanhatalo et al. 2007). It should be acknowledged that the test is very physically challenging to complete and therefore requires a highly motivated individual. If the individual is not highly motivated and/or does not perform an “all-out” sprint, the test cannot be considered valid.

The test-retest reliability of a 3MT performed on a cycle ergometer has been reported to have a standard error of estimate (SEE) of 4.8 W and 7 W or a CV% of 3 % and 3.5 % for EP (Burnley et al. 2006b; Constantini et al. 2014) while WEP is more variable (1.8 kJ 12%) (Constantini et al. 2014) or, when assessed against W′ or CP measured using the conventional method, 2.8 kJ (12%) and 6 W (2%) respectively (Vanhatalo et al. 2007).

However, it should be noted that as large as 15 W (7%) and 1.46 kJ (21%) variability has been shown in CP and W′ respectively (Johnson et al. 2011).

## 2.3 Fatigue

Athletic success is often determined by the ability to sustain a high power output or velocity throughout the duration of an event. Fatigue manifests as an inability to generate the power output required for a given task, despite maximal voluntary effort (Enoka and Duchateau 2008). Fatigue has been investigated extensively and many factors are found to contribute (Amann and Calbet 2008; Brooks 2018; Enoka and Marcora et al. 2009; Noakes 2000; Stuart 1992) including physiological, biomechanical, and psychobiological (Abbiss and Laursen 2005; Noakes 2000). There is no known single cause of fatigue but rather a combination of mechanisms are likely responsible (Abbiss and Laursen 2005; Barry and Enoka 2007). The psychobiological model of fatigue and limitations that occur within the muscle itself (Bergström and Hultman 1967; Marcora et al. 2009) will be discussed further.

Muscle contraction requires a signal from the brain to be transmitted to the contractile units of the muscle. Muscle force may be impaired by a disturbance at various sites between the brain and muscle. A disturbance prior to the neuromuscular junction denotes central fatigue (discussed in 2.4.1) whereas peripheral fatigue (discussed in 2.4.2) is defined as a process occurring distal to the neuromuscular junction (Gandevia 2001).

### 2.3.1 Overview of central fatigue

Central fatigue resides within the central nervous system (CNS) and is defined as a progressive decline in output or drive from the motor cortex to the muscle (Amann and Calbet 2008; Gandevia 2001). The afferent neurons relates exercise induced metabolic perturbation (i.e. changes in blood flow and temperature) within the muscle and sends the information back to the CNS in order to protect the organism from homeostatic imbalance (Abbiss and Laursen 2005; Amann 2011). Elevated firing of muscle afferents reduces motor neuron firing and results in an inability to sustain a power output associated with the exercise task (Amann 2011; Amann and Calbet 2008; Taylor et al. 2016). Thomas et al. (2015) showed that there is a greater degree of central fatigue during longer (>30min) compared to shorter (6 min) exercise trials, similar results has been reported on longer duration exercise as well, where subjects experienced central fatigue after 3 h of cycling (Nybo 2003). Additionally, Burnley et al. (2009) reported that during single-leg equivalent of a 3MT, torque declines concomitantly with central fatigue development and in a subsequent study demonstrated that critical torque represents a critical threshold for neuromuscular fatigue development (Burnley et al. 2012).

Many models of central fatigue exist, such as the psychological/motivational model (Abbiss and Laursen 2005). The psychological/motivational model is defined by the lack of enthusiasm or interest in exercise performance, altering central activation and perceived exertion leading to premature fatigue which will be discussed further below (Hampson et al. 2001).

#### 2.3.1.1 Mental fatigue and its effect on exercise performance

Mental fatigue is a change in one's psychobiological state caused by prolonged performance of a challenging cognitive activity (Marcora et al. 2009; Martin et al. 2015; van der Linden et al. 2006). Some studies have demonstrated a decrease in time-to-exhaustion and self-paced exercise performance after conducting various forms of mental fatiguing tasks (Brownsberger et al. 2013; Marcora et al. 2009; Pageaux et al. 2014). However, others show no effect on exercise performance (Martin et al. 2015; Pageaux et al. 2013; Pageaux et al. 2015; Van Custem et al. 2017), the power-duration relationship (Martin et al. 2015) or on neuromuscular function (Rozand et al. 2014). As little as 3 min and 40 s of a Stroop task (a cognitive task implemented to enforce mental fatigue) has been used to induce mental exertion (Bray et al. 2008), although 30-min cognitive tasks are used more frequently (Pageaux et al. 2014; Pageaux et al. 2015; Smith et al. 2016). Underlying mechanisms behind the decrements in exercise performance caused by mentally fatiguing tasks are uncertain, although it has been speculated that a greater level of perceived exertion might be key (Brownsberg et al. 2013; Marcora et al. 2009; Pageaux et al. 2014; Pageaux et al. 2013).

Studies investigating the effects of mental fatigue and mental exertion on exercise performance have mostly been conducted on recreationally active individuals (Brownsberger et al. 2013; MacMahon et al. 2014; Marcora et al. 2009; Martin et al. 2015; Pageaux et al. 2013; Pageaux et al. 2014; Smith et al. 2015). Marcora et al. (2009) reported that 90 min of a cognitive task prior to exercise decreased time-to-exhaustion from 12.6 to 10.6 min on a cycle ergometer when exercising at 80% of $\dot{V}O_{2peak}$ in recreational active individuals. Similarly, Pageaux et al. (2014) found that 30

min of mental exertion decreased average running speed during a 5-km self-paced TT in recreationally active individuals. In contrast, Martin et al. (2015), found that 90 min of a mental fatigue task had no significant effect on W′ or CP measured from a 3MT. The physiological consequences of mental stress (i.e. increased heart rate and cortisol stress-hormone levels) have been shown to be attenuated in trained individuals compared to untrained individuals (Kubitz 1993; Rimmele et al. 2007). Given the disparate physiological responses to mental stress, trained individuals are likely to cope with mental fatigue more effectively than untrained individuals. Martin et al. (2016) found that professional road cyclists did not experience performance impairment in a 20-min TT following a 30-min mentally fatiguing task while recreationally active individuals did show a decrease in TT performance compared to control. Similarly, Van Cutsem et al. (2017) conducted an experiment on endurance trained male athletes, who did not experience an impaired TT performance after performing a 45-min mentally fatiguing task compared to a control condition, in the heat.

The anterior cingulate cortex (ACC) is an area of the prefrontal cortex of the brain that is activated during cognitive stress and is associated with perception of effort (Williamson et al. 2001, 2002). The ACC plays an important role in decision making and is activated during different mentally fatiguing tasks (Lorist et al. 2005). Mental fatigue limits exercise performance through higher perception of effort suggesting that the ACC is involved in the performance decrements observed after mentally fatiguing tasks (Marcora et al. 2009). Previous research has shown that a Stroop task activates the ACC measured by increased blood flow to the prefrontal area (Ehlis et al. 2005) and that aerobic training increases the volume of the ACC, resulting in greater ACC brain

activation and cognitive control (Colcombe et al. 2006). Pacing strategies utilise the prefrontal cortex to control the conscious variation of workloads in order to limit premature fatigue (Krawczyk 2002). At the onset of self-paced exercise, cerebral $O_2$ increases as a result of a localized increase in blood flow to support the enhanced $O_2$ demand from neuronal activation (Santos-Concejero et al. 2015). It could be speculated that the reason trained athletes do not experience a decrease in exercise performance when mentally fatigue could be due to the increased ACC activation and cognitive control.

Collectively, these findings demonstrate that there a controversy as to how psychological factors affect exercise performance and uncertainty concerning the mechanisms responsible for the possible decrement in exercise performance. Likewise, what role training status has on exercise performance after prolonged cognitive functioning task is unclear.

### 2.3.2 Overview of peripheral fatigue

There are many factors associated with peripheral fatigue such as the depletion of intramuscular PCr and muscle glycogen, as well as the accumulation of $H^+$, ADP, $P_i$ and lactate (Abbis and Laursen 2005; Amann and Calbet 2008; Brooks 2018; Jones et al. 2008) which will be explained further in detail below.

#### 2.3.2.1 Intramuscular pH

To support the high demand of ATP during exercise, glucose and muscle glycogen are broken down within the muscle fiber via glycolysis. Specifically, glucose and glycogen are broken down into glucose-6-phosphate (G-6-P) via hexokinase or through

glycogenolysis, respectively. G-6-P is then catalyzed by several different enzymes with the main rate-controlling enzyme being phosphofructokinase (PFK), after which pyruvate is produced (Ferguson et al. 2018; Fitts 1994). To support ATP demand in the absence of sufficient oxygen, an accelerated rate of glycolysis is required to produce pyruvate. Excess pyruvate is catalyzed by lactate dehydrogenase (LDH) and converted to lactate (Rogatzki et al. 2015). Muscle lactate is accumulated at a faster rate in glycolytic fibers (type II muscle fibers). It is now known that the highly oxidative type I muscle fibers, as well as the brain, heart, kidneys and the liver, consume lactate as a source of fuel (Brooks 2001; Fitts 1994). Specifically, lactate can be exchanged from type II fibers to type I fibers or transported to the blood where it is picked up by the heart, brain, liver or kidneys. When type II muscle fibers produce lactate some gets released into the interstitial fluid where it is taken up by type I fibers. Lactate is thereafter converted back into pyruvate and then used within the mitochondrion (similar process happens within the brain between astrocytes and neurons). The lactate that is transported into the blood and picked up by the liver, kidneys or heart is converted back into glucose via pyruvate. The liver then either store the glucose as glycogen or transport the glucose back into the blood, while the heart and kidney use it to spare glucose within the blood (Brooks 2018; Draoui and Feron 2011). This has dispelled the assertion that the production of lactate is the key mechanism in fatigue.

The production of pyruvate from glycolysis results in an increase in $H^+$ which is diffused into the blood. Likewise, during high intensity exercise lactate acid is produced from glycolysis and split into lactate and $H^+$. In the blood, $H^+$ reacts with sodium bicarbonate into sodium and carbonic acid and further dissociates into water and $CO_2$ via the

enzyme carbonic anhydrase. However, if the rate of muscle $H^+$ production exceeds the rate of disposal, acidosis results. A fall in muscle pH from 7.0 to as low as 6.2 has been shown to inhibit cross bridge cycling (Abbis and Laursen 2005; Debold et al. 2016; Fabiato and Fabiato 1978; Fitts 1994; Fitts 2008). Specifically, this has been demonstrated to reduce unloaded shortening cycle velocity by 20-30% in skinned muscle fibers as well as slowing actin filament velocity by 67% (Debold et al. 2016). The increase in $H^+$ causes a decrease in glycolytic flux by inhibiting PFK and disturbs muscle contractility by reducing myofibrillar $Ca^{2+}$ sensitivity (Abbiss and Laursen 2005; Debold et al. 2016; Hill et al. 2001). The decreased $Ca^{2+}$ sensitivity is thought to be one of the main reasons for the loss in isometric force during fatiguing exercise (Debold et al. 2016). Therefore, during severe intensity exercise the increase in $H^+$ can indirectly be responsible for the fatiguing process in this domain.

Even though there is evidence that acidosis causes fatigue it should be acknowledged that there are studies opposing this. Specifically, several studies have failed to find a correlation between muscle function and acidosis, such as it has been shown that force recovers more rapidly than pH at the end of fatiguing contractions (Degroot et al. 1993; Ferguson et al. 2018; Sahlin and Ren 1989; Westerblad and Allen 1992). Correspondingly, the number of tetani required to induce muscle fatigue in single mouse muscle fibers has little effect on acidification at near physiological temperatures, indicating that the fatigue development does not seem to be accelerated by acidosis (Bruton et al 1998; Westerblad et al. 2002). Therefore, other mechanisms which may (also) be responsible for impaired muscle functioning will be discussed below.

### 2.3.2.2 Accumulation of extracellular $K^+$

The transmission of an action potential across the muscle membrane to the t tubules of a muscle fiber is dependent upon sodium ($Na^+$) and potassium ($K^+$) concentrations within the cell. Disproportionate and unregulated $Na^+$ or $K^+$ flux prevents ongoing muscle contraction (Fitts 1994; Kuo and Ehrlich 2015; McKenna et al. 2007). During repeated muscle activation, a net $K^+$ efflux from the muscle cell occurs leading to an increase in extracellular $K^+$ concentrations at the expense of intracellular $K^+$ (Clausen 2003; Juel et al. 2000; Sjogaard 1985). Increasing extracellular $K^+$ impairs force generation due to the depolarization of the cell membrane which in turn will inactivate $Na^+$ channels and reduce the amplitude of the action potential. The accumulation of extracellular $K^+$ during prolonged membrane depolarization reduces SR $Ca^{2+}$ release, impairing excitation-contraction coupling (McKenna 1992).

### 2.3.2.3 Muscle PCr and $P_i$ concentrations

To maintain cross bridge cycling during submaximal exercise there is a great reliance on ATP production via oxidative phosphorylation (Allen et al. 2008). However, PCr hydrolysis contributes to the production of ATP necessary to sustain severe-intensity exercise (Westerblad et al. 2002). Therefore, during severe-intensity exercise there is a marked decrease in muscle PCr and as a result, an increase in $P_i$ (Jones et al. 2008; Westerblad et al. 2002).

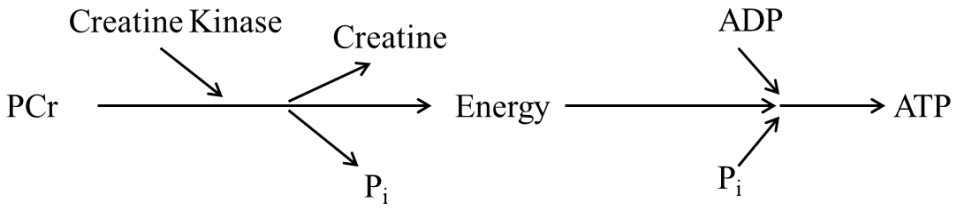

***Figure 2.5**. Schematic representation of the hydrolysis of PCr.*

Investigators have established that $P_i$ can cause fatigue by “knocking” the myosin head off actin (Debold et al. 2016; Debold et al. 2013). High levels of $P_i$ within the myoplasm force myosin to detach from actin at an earlier stage before the power stroke has finished (Debold et al. 2016). $P_i$ is also implicated in reducing SR $Ca^{2+}$ uptake and decreasing available $Ca^{2+}$ for SR release (Westerblad et al. 2002). It has been shown that $P_i$ enters the SR resulting in a $Ca^{2+}$-$P_i$ precipitation thus reducing the $Ca^{2+}$ availability for release (Allen et al. 2008; Debold et al. 2016). Furthermore, the accumulated myoplasmic $Ca^{2+}$ may leave the cell and reduce the $Ca^{2+}$ available to sustain contraction (Allen and Westerblad 2001; Westerblad et al. 2002). Moreover it has been shown that much of the loss in isometric force is due to the increased levels of $P_i$ (and $H^+$), reducing $Ca^{2+}$ sensitivity, which causes a right shift in the force-calcium relationship which means that more $Ca^{2+}$ is required to activate the filaments (Debold et al. 2006; Debold et al. 2016).

#### 2.3.2.4 Muscle glycogen concentration

Muscle glycogen depletion is a key feature of long-duration endurance exercise (Coyle et al. 1986; Gigli and Bussmann 2002; Jeukendrup et al. 2004). During endurance events, glycogen and glucose are utilized to produce ATP via oxidative phosphorylation and substrate level phosphorylation (discussed in chapter 2.3). Glucose is readily available in the blood for the muscle to use as energy while glycogen stored within the muscle and liver are mobilized to support energy provision (Abbiss and Laursen 2005). Extremely low glycogen and glucose availability results in a shift to lipid metabolism (Abbiss and Laursen 2005; Jeukendrup 2005). However, the, oxidation of fat (2.0092

$L \cdot g^{-1}$ $O_2$) incurs a greater $O_2$ cost per unit of work compared to glucose (0.7455 $L \cdot g^{-1}$ $O_2$) and glycogen (0.8283 $L \cdot g^{-1}$ $O_2$) (Bergström and Hultman 1967; Jeukendrup and Wallis 2005).

Muscle glycogen is utilized at high rates during severe-intensity exercise; however muscle glycogen depletion is not the cause of exhaustion (Brooks 1997; Hawley et al. 1997; Rockwell et al. 2003; Stepto et al. 2001). Rather, the critically low values of PCr and pH and high values of $H^+$, ADP and $P_i$ (described above) are associated with the limit of tolerance during severe-intensity exercise (Black et al. 2017; Burnley et al 2010; Hogan et al. 1999; Jones et al. 2008). In contrast, prolonged (>90 min) exercise at intensities equivalent to 70-77% of $\dot{V}O_{2max}$ are associated with critically low values of muscle glycogen (Bergström et al. 1967; Bosch et al. 1993; Hermansen et al. 1967; Hawley et al. 1997; Black et al. 2017). Black et al. (2017) reported that exercising for ~3.5 h caused minor muscle metabolic perturbation (i.e. PCr, pH, plasma [$K^+$] and blood [lactate]) but a large reduction in muscle glycogen and concluded that the key mechanism for decline in neuromuscular function during moderate exercise was related to the depletion of muscle glycogen. While Black et al. (2017) did not analyses the response of specific muscle fibers, it is anticipated that the muscle glycogen depletion is present in both type I and II muscle fibers but the reduction in muscle glycogen occurs to a greater extent in type I muscle fibers (Gollnick et al. 1974). Indeed, after 2-h of exercise at 64-70% $VO_{2max}$ both type I and type II muscle fibers exhibit glycogen depletion, but at 95% and 53% respectively (Tsintzas and Williams 1998; Gollnick et al. 1973; Gollnick et al. 1974). Therefore, during 2 h of heavy-intensity exercise the depletion of muscle glycogen in almost completed within type I muscle fibers and a

subsequent exercise bout would need to be maintained by the muscle glycogen residing within the type II muscle fibers.

The low muscle [glycogen] observed during long duration activity is also associated with a reduction in SR $Ca^{2+}$ release rate (Chin and Allen 1997; Gejl et al. 2014). Low muscle [glycogen] induced by prior diet restriction and prolonged exercise to exhaustion (66.7 ± 59 min) at 70 % $\dot{V}O_{2peak,}$ is associated with an earlier reduction in SR $Ca^{2+}$ uptake and $Ca^{2+}$ release, compared to when glycogen levels are maintained high (Duhamel et al. 2006). In addition, under conditions when myoplasmic ATP and PCr (exposed to a polarizing solution) are held high and constant, reduced muscle contractile function and fatigue resistance is correlated to low muscle [glycogen] (Nielsen et al. 2009). It is therefore likely that muscle glycogen depletion is a key mechanism underlying peripheral fatigue during prolonged endurance activity.

It is known that muscle glycogen availability plays an imperative role in exercise lasting longer than 90 min, but no evidence is available to show how CP or W' might change during prolonged exercise. Miura et al. (2000) investigated the effects of glycogen depletion in form of dietary restriction on the power-duration relationship. While no differences in CP were observed between a glycogen depleted and ‘normal’ state, W' was significantly decreased. However, it remains unclear how the depletion of muscle glycogen as a result of prolonged fatiguing exercise impacts the power-duration relationship.

### 2.3.2.5 Carbohydrate supplementation and its role in exercise performance

An increasing evidence base supporting the crucial role of muscle glycogen during prolonged exercise and importantly, for carbohydrate feeding to extend time to exhaustion, has fueled several investigations into the mechanisms responsible (Coyle et al. 1986; Dennis et al. 1997; Gigli and Bussmann 2002; Hermansen et al. 1967; St Clair Gibson 2001). Of these mechanisms, emerging evidence suggests that carbohydrate feeding maintains high levels of carbohydrate oxidation (Coyle et al. 1983; Howlett et al. 1998; Jeukendrup et al. 1999), thereby sparing endogenous glycogen use (Bosch et al. 1993; Coyle et al. 1986), while also stimulating the central nervous systems (Carter et al. 2004a; Carter et al. 2004b). In events lasting ~1 h (>75% of $\dot{V}O_{2peak}$), muscle glycogen is not fully depleted (Black et al. 2016; Jeukendrup 2004). Nonetheless the consumption of carbohydrate increases time trial (TT) cycling performance (Jeukendrup et al. 1997) and distance covered during ~1 h running (Rollo and Williams 2009). New research suggests an immediate benefit of carbohydrate ingestion due to the similar improvements in exercise performance observed following carbohydrate mouth rinse (Bastos-Silva et al. 2016; Chambers et al. 2009; James et al. 2017; Murray et al. 2018). James et al. (2017) showed that when using carbohydrate mouth rinse, subjects completed a TT ~2 min (~57 min versus ~59 min) faster compared to a placebo condition. Similarly, Bastos-Silva et al. (2016) showed that when cycling at 80% of the respiratory compensation point, subjects lasted another ~11 min (~76 min for carbohydrate versus ~65 min for placebo) when receiving carbohydrate mouth rinse compared to a placebo. The mechanisms responsible for the increase in performance during mouth rinsing with a carbohydrate solution are not fully established. However, it

is believed that performance improvements are likely related to an increase in corticomotor excitability and central motor drive to the exercising muscle (Carter et al. 2004; Gant et al. 2010). Carbohydrate feeding may serve as a positive afferent signal capable of modifying motor output (Jeukendrup 2011). Chambers et al. (2009) found that specific regions of the brain (insula/frontal operculum, oribitofrontal cortex and stratum) are activated when carbohydrates enter the oral cavity, independent of sweetness. These specific regions of the brain are believed to be associated with reward and sensory perception, which in turn could influence behavioral responses (Kringelbach et al. 2004; Turner et al. 2014).

Consuming carbohydrates during longer events (lasting more than 2-h) can improve exercise performance and exercise tolerance (Coggan and Coyle 1988). The maximal rate that ingested glucose can be oxidized is ~1.0 $g \cdot h^{-1}$, but when combined with other carbohydrate sources such as fructose, the carbohydrate oxidation rate can increase to that above ~1.0 $g \cdot h^{-1}$ (Jeukendrup 2004). If a larger volume is ingested there is a possibility of gastrointestinal distress. Pfeiffer et al. (2009) reported a greater likelihood of nausea when consuming 90 $g \cdot h^{-1}$ of carbohydrate compared to 60 $g \cdot h^{-1}$. However, gastrointestinal problems during exercise vary highly between modes of exercise (Pfeiffer et al. 2012). Ingestion of 60 g of carbohydrate per hour during a 2-h constant work rate bout at 77% of $\dot{V}O_{2max}$ has been shown to increase a subsequent TT performance (Smith et al. 2010b). Furthermore it has also been reported that endurance trained cyclists exercising at 71% of $\dot{V}O_{2peak}$ for 2 h with concurrent carbohydrate supplementation (2 $g \cdot kg^{-1}$ body weight of a glucose polymer at 20 min and 0.4 $g \cdot kg^{-1}$

body weight every 20 min thereafter), extended time to exhaustion by up to an hour in a subsequent constant work rate bout at the same intensity (Coyle et al. 1986).

Collectively, these findings demonstrate that the administration of carbohydrates through ingestion or in the form of a mouth rinse, prolong time to exhaustion during long-duration exercise. Given that exercise >90 mins results in large reduction in muscle glycogen content, muscle glycogen depletion may negatively affects the parameters of the power-duration relationship with a possibility that carbohydrate supplementation may attenuate the possible changes in CP and W' induced by prolonged exercise.

## 2.4 Influence of fatigue on the power-duration relationship

Some studies have investigated the effects of prior severe-intensity exercise on CP and W' (Ferguson et al. 2007; Parker Simpson et al. 2012; Vanhatalo and Jones 2009). Vanhatalo and Jones (2009) investigated the effect of 30-s prior sprint exercise with variable recovery periods on CP and W' estimated from a 3MT. It was shown that when a 2-min recovery was administered after the 30-s sprint, there was a significant reduction in W' but not CP. The 30-s sprint depleted W' by 65% and the 2-min recovery restored W' to 79% of the resting value. However, when a 15-min recovery was applied there was no difference in either parameter. This suggests that 15 min of recovery is sufficient to restore W' following a 30-s all-out sprint. Studies have demonstrated similar findings when investigating the effects of prior exercise within the severe-intensity domain on estimates of CP and W'. Ferguson et al. (2007) found that 6 min of exercise in the severe-intensity domain with 2 min of rest prior to the estimation of CP and W' using the conventional prediction trial protocol did not affect CP but decreased W' by

35%. Similarly, Parker Simpson et al. (2012) found that exercising for 2 and 4 min within the severe-intensity domain prior to a 3MT decreased W' by 33% and 48% respectively, but did not alter CP. It is thought that the depletion and restoration of PCr is the key determinant of W' in these studies (Vanhatalo and Jones 2009). During all-out exercise the peak power output is dependent on PCr content, specifically it has been shown that PCr content can fall to 20% of resting values during a 30 s of all out sprinting (Bogdanis 1995, 1996; Chettham et al. 1986).

Jones et al. (2003) investigated the effect of prior heavy-intensity exercise on CP and W'. In contrast to the effects reported following severe-intensity exercise, 6 min of prior heavy-intensity exercise followed by a 10 min recovery period did not alter CP but significantly increased W'. Likewise, Burnley et al. (2011) also found that 6 min of heavy-intensity exercise followed by a 10 min recovery period increased the W' but did not alter CP, while 6 min of severe-intensity exercise followed by the same 10 min recovery period had no effect on CP or W'. The increase in W' following heavy-intensity exercise was likely due to a priming effect on $\dot{V}O_2$ kinetics, i.e., an increase in $\dot{V}O_2$ amplitude (increase above baseline values) with no change in time constant and a reduced slow component, resulting in an increased aerobic contribution to energy metabolism such that the spared substrate level phosphorylation could be used during the prediction trials (Burnley et al. 2002, 2006a, 2011; Jones et al. 2003, 2008). In addition, heavy-intensity exercise does not progressively deplete PCr or cause metabolite accumulation, and the 10 min recovery period was sufficient to restore resting concentrations (Burnley et al. 2011). Parker Simpson et al. (2012) found that CP and W' were unaffected following exercise for 6 min at intensities within the moderate-

and heavy-intensity domain immediately prior to a 3MT. These studies show that W' is a reserve that is accessed when power output exceeds CP and therefore is unaffected by prior exercise at power outputs that are below CP (Ferguson et al. 2007; Parker Simpson et al. 2012; Vanhatalo and Jones 2009). It should be noted that the time spent within the moderate- and heavy-intensity domains in these studies did not exceed 6 min. It is therefore unknown how CP and W' might be affected by prolonged exercise that is associated with significant muscle glycogen depletion.

To date, the effect of prolonged, fatiguing exercise on CP and/or W', and subsequently on the prediction of exercise performance using these parameters is unknown. CP is strongly related to the fatigue-resistant type I muscle fiber type composition and cross sectional area of type I muscle fibers, demonstrating further that CP is an oxidative metabolic parameter (Mitchell et al. 2018; Vanhatalo et al. 2016). At the start of all-out exercise the least fatigue resistant type II fibers are used to sustain the required power output, thereafter there is a drop out of type II muscle fibers (seen by the progressive decline in power output) as all-out exercise continues (McCartney et al. 198). After 2-3 min of all-out sprinting the plateau in maximal power output is likely to be sustained by type I muscle fibers, as CP reflects external work generated primarily by the type I muscle fibers (Vanhatalo and Jones 2009). Consequently, it can be speculated that if the type I muscle fibers have been excessively used during prolonged fatiguing exercise CP will be decreased. Previous research has shown that W' is decreased after sprinting as well as in a glycogen depleted state (Ferguson et al. 2007; Miura et al. 2000; Parker Simpson et al. 2012; Vanhatalo and Jones 2009). Therefore it is anticipated that there would also be a reduction in W' following prolonged fatiguing exercise <CP.

## 2.5 Summary

In summary, it is possible that both physiological and psychological factors regulate the limits of exercise performance and that the relative contribution of these factors on performance may influence the power-duration relationship and differ between trained and untrained individuals. The parameters of the power-duration relationship, CP and W', have significant value in exercise performance prediction. Knowledge of CP and W' can inform predictions of performances over given distances and durations. In turn, this knowledge can inform coaches and athletes when formulating race strategies that elicit the best possible performances. However, it is possible that CP and W' are altered during long-duration events by factors relating to fatigue. Therefore, the primary focus of this thesis was to investigate key contributing factors to fatigue and how these impact upon the parameters of the power-duration relationship. A greater understanding of how the power-duration parameters might change with exercise duration will have value for the prediction of exercise performance during long duration exercise.

## 2.6 Aims and hypothesis

### 2.6.1 Aims

The aim of the thesis was twofold. Firstly, to investigate the possible psychobiological factors contributing to fatigue in competitive athletes and, secondly, to investigate the effect of prolonged fatiguing exercise on the power-duration parameters. The specific research questions addressed are:

1) How does a prolonged cognitive task affect subsequent exercise performance in untrained individuals and competitive athletes?

- Is untrained or competitive athletes' exercise performance affected by a prolonged cognitive functioning task?

2) What are the effects of 2 h of fatiguing heavy-intensity exercise on CP and W'?
   a) Is the 3MT a reliable test for estimating EP and WEP when administered in a fatigued state?
   b) Does EP estimated following 2 h of heavy-intensity exercise demarcate the heavy and the severe exercise domains?
   c) Do the CP and W' estimates derived from the 3MT and the conventional prediction trial protocols differ after 2 h of heavy intensity exercise?
3) What is the time course over which EP and WEP deteriorate during prolonged endurance exercise?
4) Does carbohydrate feeding eliminate possible changes in CP and W' after 2 h of heavy-intensity exercise?

### 2.6.2 Hypotheses

The thesis will address the following hypotheses:

1. A prolonged cognitive task performed before exercise would decrease the amount of work completed during a TT in untrained individuals but not in competitive athletes. Furthermore, frontal-lobe activation would be greater in the prolonged cognitive tasks condition compared to a control condition for untrained individuals, but not competitive athletes.
2. Following 2 h of heavy-intensity exercise, the 3MT would provide reliable EP and WEP values. EP and WEP estimated after 2 h of heavy intensity exercise would be significantly lower compared to values derived from a 3MT completed with no

prior exercise. EP estimated after 2 h of heavy-intensity exercise would demarcate the heavy and severe exercise intensity domains, as defined by an inability to stabilize blood [lactate] and the development of a $\dot{V}O_2$ slow component leading to the attainment of $\dot{V}O_{2peak}$, during exercise above, but not below, EP.

3. Following 2 h of heavy intensity exercise, fatigued CP (F-CP) and fatigued W' (F-W') estimated using the conventional prediction trial protocol would not be different from the fatigued EP (F-EP) and fatigued WEP (F-WEP) estimated using the 3MT. Furthermore, F-CP and F-W' estimated using the conventional protocol would be significantly lower compared to those estimated in a rested state.
4. Forty min and 80 min of prior heavy intensity exercise would significantly reduce CP and W'. Supplementing with a CHO beverage would attenuate the decrease in CP and W' observed after the consumption of a placebo beverage during 2 h of heavy intensity exercise.

# Chapter 3: General methods

## 3.1 Health and safety

All exercise testing was conducted in an exercise physiology lab at sea level. All experimental procedures in each experimental chapter were approved by the University of Exeter research ethics committee prior to recruitment or data collection. All testing procedures were in line with the health and safety guidelines established by the Sport and Health Sciences Department at the University of Exeter. The equipment was thoroughly cleaned using Virkon disinfectant prior to each participant entering the facility. All respiratory equipment were disinfected according to manufactures guidelines. During collection of blood or muscle biopsies, researchers wore sterile gloves and disposable laboratory coats. All biohazardous materials were disposed of appropriately and biopsy needles were sterilized prior to use. All participants who underwent a biopsy procedure were provided with a post-procedure care information sheet.

## 3.2 Ethical approval and informed consent

Prior to testing, participants were provided with a written subject information sheet outlining the procedures of the study, after which they were given a verbal explanation of the procedures and given the opportunity to ask questions. Participants were informed that they could withdraw from the study at any point throughout the experiment with no disadvantage to themselves and that their data would be anonymously published in academic journals and safely stored. Participants completed a physical activity readiness questionnaire (PAR-Q). If the participant fulfilled the inclusion criteria

to partake in the study, and provided that the participant was willing and able to comply with the study protocols, they provided their written informed consent.

### 3.3 Participants

Volunteers recruited for testing were from the University of Exeter student population and from the local community. Volunteers recruited for chapter 4 were either from the student population or competitive athletes from various sports clubs in the Exeter area. Volunteers for chapters 5-7 were enrolled in the study if they were familiar with long duration cycling exercise prior to the study.

All participants were non-smokers, free of any disease, and did not use any dietary supplements or medication at the time of data collection. Participants were asked to maintain normal exercise and diet habits throughout their participation. Volunteers for chapter 4 recorded their exercise regimen for 7 days leading up to each scheduled testing day. Participants for chapter 5-7 recorded their diet and exercise regimen 24 h prior to each testing day to ensure reproducibility throughout the study. All exercise testing for a given participant took place at the same time of day (±2 h) and all participants were familiarised with the exercise procedures prior to testing. Before arriving to the laboratory for experimental visits, participants were asked to avoid strenuous exercise and the consumption of alcohol for 24 hours as well as to avoid caffeine for 2 hours.

### 3.3. Cycle ergometry

All the exercise tests were conducted using the same electrically-braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). For each participant, the ergometer seat and handlebar configuration were adjusted for comfort during the first visit and replicated for subsequent visits. The experimental chapters use a combination of hyperbolic, step and linear work forcing functions of the cycle ergometer. The hyperbolic function was used during the ramp incremental protocol allowing the work rate to increase linearly over time. The step function was used when a constant work rate was administered. This function allows for a near instant change in work rate (1 000 $W \cdot s^{-1}$) in a step wise manner and is independent of cadence. The linear function was used for the TT in chapter 4 and for all 3MTs administered throughout the experimental chapters. The linear function imposes work rate based on cadence with use of the following equation:

$$\text{Linear factor} = \text{Power output}/\text{Cadence}^2 \quad [d]$$

By using the linear function, the ergometer is set to a fixed resistance so that the attainment of a particular cadence will provide a known power output. The ergometer was regularly calibrated and serviced by a qualified technician according in accordance with the manufacturer's guidelines.

### 3.4 Ramp incremental test

The ramp incremental test was preceded by a 3 min baseline cycling at 20 W. Thereafter, power output increased linearly by 30 $W \cdot min^{-1}$ (except chapter 4 in which 25 $W \cdot min^{-1}$ or 35 $W \cdot min^{-1}$ were used for the untrained or athletic population respectively).

Prior to the start of the visit, participants were allowed to select a desired cadence between 70-90 revolutions per minute (rpm) which they were instructed to hold throughout the entire test. The limit of tolerance was determined when cadence fell more than 10 rpm below the target cadence for more than 5 s despite strong verbal encouragement.

Breath-by-breath pulmonary gas exchange data were collected continuously throughout all incremental ramp tests. $\dot{V}O_{2peak}$ was determined as the highest 30 s mean value recorded during the test. The GET was defined as: 1) the first disproportionate increase in $\dot{V}CO_2$ relative to $\dot{V}O_2$; and 2) an increase in the ventilatory equivalent for $O_2$ ($\dot{V}E/\dot{V}O_2$) with no increase in the ventilatory equivalent for $CO_2$ ($\dot{V}E/\dot{V}CO_2$). To account for the lag in the $\dot{V}O_2$ during the incremental test, two-thirds of the ramp rate (i.e., 2/3 x 30 W = 20 W) was deducted from the power output at GET and the peak power output attained in the test (Davis et al. 1982; Whipp et al. 1981).

### 3.5 Normalising exercise intensity

In chapter 5-7 participants were required to exercise within the heavy-intensity domain for the duration of their fixed constant work rate bouts. Therefore the GET obtained from the ramp incremental test as well as the CP from the 3MT (performed in a rested state) were used to calculate 25%$\Delta$1, which refers to a power output at the GET plus 25% of the difference between the GET and CP. Based on pilot work, the power output at 25%$\Delta$1 was within the heavy-intensity domain and sustainable for 2 h.

### 3.6 The 3-min all-out exercise test

Each 3MT was preceded by a period of cycling at 20 W. All 3MTs administered in a rested state were preceded by a 3 min period of cycling at 20 W, while each 3MT following a constant work rate bout were preceded by 30 s of cycling at 20 W. Each participant had a 10 s countdown before the start of the 3MT. During the final 5 s, the participant was prompted to increase their cadence to 110-120 rpm. At the word ‘go’ the participant sprinted in an all-out effort, which was held for a total of 3 min, with strong verbal encouragement given throughout. To prevent pacing, no feedback of time elapsed was given to the participant during the 3MT. At the end of the 3MT, subjects were allowed a cooldown and were monitored until they felt ready to leave the laboratory.

When administering a 3MT on a cycle ergometer, a fixed resistance is required for the subject to cycle against. The resistance for the 3MTs is calculated using a linear factor specific to the Lode Excalibur Sport ergometer (equation d) where the power is 50%Δ (work rate eliciting GET plus 50% of the difference between work rate at GET and $\dot{V}O_{2max}$) measured in Watts and preferred cadence is the rpm used during the preliminary ramp incremental test (Burnley et al. 2006). Power output was recorded every 0.2 seconds and averaged out as second by second values. For a test to be deemed valid 95% of $\dot{V}O_{2peak}$ had to be achieved and maintained together with no pacing (i. e. increase in power output at any point). Peak power output was calculated as the highest 1 second value. CP is estimated from the 3MT by averaging the power output over the last 30 s of the test and the W' is estimated by calculating work performed above CP using:

$$W' = (P_{150} - CP) \times 150 \text{ s} \quad [e]$$

where W' is measured in joules, $P_{150}$ is the average power output (Watts) over the first 150 s of the test, CP measured in Watts.

### 3.7 Pulmonary gas exchange

During all studies pulmonary gas exchange was measured breath-by-breath and averaged over 10-s intervals. In chapter 4 and 5, participants wore a nose clip and breathed through a low dead space (90 mL), low resistance (0.75 mm $Hg \cdot L^{-1} \cdot s^{-1}$ at 15 $L \cdot s^{-1}$) mouthpiece and impeller turbine assembly (Jaeger Triple V). The inspired and expired gas volume and gas concentration signals were sampled continuously at 100 Hz (Jaeger Oxycon Pro, Hoechberg, Germany) via a capillary line connected to the mouthpiece. The analysers were calibrated before each test with gases of known concentrations and the turbine volume transducer was calibrated using a 3 L syringe (Hans Rudolph, MO). During chapter 6 and 7 participants wore an oro-nasal mask (Hans Rudolf 7450 Series V2$^{TM}$ Mask, CareFusion, Germany) and the inspired and expired gas volume and gas concentration signals were sampled continuously at 100 Hz (Vyntus, CareFusion, Germany) via a capillary line connected to the mask. These analysers were calibrated before each test with gases of known concentration and the turbine volume transducer was calibrated using a 3 L syringe (Hans Rudolph, MO). During all studies the volume and concentration signals were time-aligned by accounting for the delay in capillary gas transit and analyser rise time relative to the volume signal.

### 3.8 Venous blood sampling

In chapter 4 and 6, venous blood samples were obtained at rest and during exercise. Prior to testing, a cannula (Insyte-W; Becton Dickinson, Madrid, Spain) was inserted in an antibecubital vein. All blood samples were collected into a lithium-heparin vacutainer (Becton-Dickinson, New Jersey, USA). 200 µL of blood was immediately extracted and haemolysed in 200 µL of Triton X-100 Solution (Triton X-100, Amresco, Salon, OH) and blood [glucose] and blood [lactate] were immediately measured (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). The remaining blood was centrifuged at 4000 rpm for 10 min at 4°C. In chapter 4, the plasma was analysed immediately for $Na^+$, $K^+$ (9180 Electrolyte Analyzer, F. Hoffmann-La Roche, Basel, Switzerland). The remaining plasma for both chapter 4 and 6 was extracted and frozen at -80°C for further analysis (chapter 4: cortisol; chapter 6: $K^+$, using Stat Profile pHOx Ultra, Nova Biomedical, Waltham, MA, USA). In chapter 5 – 7, fingertip blood samples (~25 µl) were collected into capillary tubes during rest (chapter 5 and 7) and during exercise. Blood samples were promptly analysed for blood [lactate] and blood [glucose] using an automated lactate analyser (YSI 2300, Yellow Spring Instrument, Yellow Springs, OH).

### 3.9 Muscle glycogen

In chapter 6 and 7 muscle samples were freeze-dried prior to dissection from connective tissue, fat and blood. Approximately 2 mg of dry weight muscle tissue was hydrolyzed in 500 µl of 1 M hydrochloric acid at 100°C for 3 h to release glycosyl units and immediately measured using an automated glucose analyser to determine muscle [glycogen] (YSI 2900 Biochemistry Analyzer; Yellow Springs Instruments, Yellow Springs, OH). The following method was also used to analyze muscle glycogen in

chapter 7. Muscle glycogen was extracted from ~1 mg d.w. muscle and hydrolysed to glucose units in 1M HCl at 95°C for 3 h. The addition of hexokinase catalyzed the reaction of glucose with adenosine triphosphate to glucose-6-phosphate, and then to 6-P-gluconolactone with NADH+ in the presence of G-6-PDH enzyme, producing the fluorescent detectable NADPH. Reactions were measured on a Fluoroskan (Fluoroskan™ Microplate Fluorometer, ThermoFisher Scientific, Mass. USA), with Excitation 355 nm and Emission 460 nm filters. Glycogen was reported in units of mmol of glucose per kg dry muscle. Comparing the 2 methods in chapter 7 did not elicit any differences, with an $R^2$ of 0.92. The latter described method was chosen for the reporting of muscle glycogen in chapter 7 due to its more frequent use in the available literature and because one subject's samples had to be excluded using the first method due to contamination.

### 3.10 Statistical analysis

Statistical analyses in all experimental chapters were done using the Statistical Package for Social Sciences (SPSS). The specific statistical procedures for each chapter are given within the statistical analysis section of each experimental chapter. All data are represented as mean ± SD if not otherwise stated. Statistical significance was accepted at $P < 0.05$.

## Chapter 4. Time-trial performance is not impaired in either competitive athletes or untrained individuals following a prolonged cognitive task

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ORIGINAL ARTICLE

# Time-trial performance is not impaired in either competitive athletes or untrained individuals following a prolonged cognitive task

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**Abstract**
It has been reported that mental fatigue decreases exercise performance during high-intensity constant-work-rate exercise (CWR) and self-paced time trials (TT) in recreationally-trained individuals. The purpose of this study was to determine whether performance is impaired following a prolonged cognitive task in individuals trained for competitive sport. Ten trained competitive athletes (ATH) and ten untrained healthy men (UNT) completed a 6-min severe-intensity CWR followed by a 6-min cycling TT immediately following cognitive tasks designed to either perturb (Stroop colour-word task and N-back task; PCT) or maintain a neutral (documentary watching; CON) mental state. UNT had a higher heart rate (75 ± 9 v. 69 ± 7 bpm; $P = 0.002$) and a lower positive affect PANAS score (19.9 ± 7.5 v. 24.3 ± 4.6; $P = 0.036$) for PCT compared to CON. ATH showed no difference in heart rate, but had a higher negative affect score for PCT compared to CON (15.1 ± 3.7 v. 12.2 ± 2.7; $P = 0.029$). Pulmonary $O_2$ uptake during CWR was not different between PCT and CON for ATH or UNT. Work completed during TT was not different between PCT and CON for ATH (PCT 103 ± 12 kJ; CON 102 ± 12 kJ; $P > 0.05$) or UNT (PCT 75 ± 11 kJ; CON 74 ± 12 kJ; $P > 0.05$). Compared to CON, during PCT, UNT showed unchanged psychological stress responses, whereas ATH demonstrated increased psychological stress responses. However, regardless of this distinction, exercise performance was not affected by PCT in either competitive athletes or untrained individuals.

**Keywords** Mental fatigue · Exercise performance · Anterior cingulate cortex · Cerebral oxygenation · Competitive athletes

**Abbreviations**

| | |
|---|---|
| ACC | Anterior cingulate cortex |
| ATH | Competitive athletic participants |
| CNS | Central nervous system |
| $CO_2$ | Carbon dioxide |
| CON | Control condition |
| [cortisol] | Plasma cortisol concentration |
| CP | Critical power |
| CWR | Constant-work-rate exercise |
| EDTA | Ethylenediaminetetraacetic-acid |
| $GET_{met}$ | Metabolic rate at gas exchange threshold |
| [glucose] | Plasma glucose concentration |
| $\Delta[HbO_2]$ | Change in concentration of tissue oxyhemoglobin |
| $\Delta[Hb_{tot}]$ | Change in concentration of tissue total hemoglobin |
| Δ[HHb] | Change in concentration of tissue deoxyhemoglobin |
| HR | Heart rate |
| INC | Incremental cycling test |
| $[K^+]$ | Plasma potassium concentration |
| [lactate] | Plasma lactate concentration |
| LH | Lithium heparin |
| MF | Mental fatigue |
| $[Na^+]$ | Plasma sodium concentration |

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| | |
|---|---|
| NIRS | Near-infrared spectroscopy |
| $O_2$ | Oxygen |
| PA-R | Physical activity rating tool |
| PCT | Prolonged cognitive task |
| PFC | Prefrontal cortex |
| PANAS | Positive and negative affect scale |
| RPE | Rating of perceived exertion |
| TOI | Tissue oxygenation index |
| TT | Time trial |
| UNT | Untrained participants |
| $\dot{V}CO_2$ | Rate of carbon dioxide output |
| $\dot{V}E$ | Rate of minute ventilation |
| $\dot{V}O_2$ | Rate of $O_2$ consumption |
| $\dot{V}O_{2peak\text{-}CWR}$ | Peak rate of $O_2$ consumption during CWR |
| $\dot{V}O_{2peak\text{-}INC}$ | Peak rate of $O_2$ consumption during INC |
| $\dot{V}O_{2peak\text{-}TT}$ | Peak rate of $O_2$ consumption during TT |
| $W_{GET}$ | Rate of work that precipitated $GET_{met}$ |
| $Work_{TT}$ | Total work completed during TT |
| $W_{peak\text{-}INC}$ | Peak rate of work during INC |

## Introduction

The factors responsible for the inability to sustain high-intensity exercise have long been debated (Contessa et al. 2016). Limiting factors might include a reduced capacity of the central nervous system (CNS) to activate required motor units and/or a decreased response of recruited muscle fibres to a given level of CNS activation (i.e. central or peripheral fatigue, respectively). However, a unifying concept is that a central or peripheral limitation of physiological origin creates the inability to maintain force/power output despite maximal voluntary effort. Challenges to this traditional model of exercise tolerance (e.g. Noakes et al. 2005) have been difficult to confirm empirically (Inzlicht and Marcora 2016).

Marcora et al. (2009) reported that time to exhaustion during high-intensity constant-work-rate (CWR) cycling was reduced following completion of a prolonged cognitive task (PCT) that required participants to engage in sustained attention, working memory, response inhibition and error monitoring for 90 min. Interestingly, this reduced ability to perform exercise was not accompanied by changes in mood, motivation or autonomic activation; however, subjective rating of perceived exertion (RPE) was increased throughout the exercise bout that followed the PCT. The authors concluded that 'mental fatigue' (MF) induced by the PCT heightened perception of physical effort independent of changes in cardiorespiratory, metabolic and/or neuromuscular responses (Marcora et al. 2009). This resulted in earlier disengagement from the exhaustive task and a corresponding inability to achieve the heart rate (HR) and blood lactate responses that were present at task failure when a neutral mental state was present (Marcora et al. 2009; Martin et al. 2018; Pageaux and Lepers 2016).

Perception of effort as a limiting factor in highly motivated participants challenges traditional notions regarding physiologically-based central and peripheral fatigue (Marcora and Staiano 2010). An ergolytic effect of MF on endurance performance may have implications for athletes both in competition and training. However, most research investigating the influence of MF on exercise performance has been conducted on individuals who performed regular physical training, but were not competitive athletes (Marcora et al. 2009; Brownsberger et al. 2013; Pageaux et al. 2013, 2014; MacMahon et al. 2014). One exception was a study involving professional road cyclists who did not experience the ergolytic effect of MF that was evident for recreational cyclists (Martin et al. 2016). Studies on non-professional competitive athletes have produced mixed results. For example, Van Cutsem et al. (2017a) found that male cyclists/triathletes did not experience impaired TT performance following MF; however, in that study, both MF and control conditions were performed in the heat (30 °C with 30% relative humidity). Consequently, the lack of effect of MF on TT performance could not be definitively ascribed to the athletic status of participants (Van Cutsem et al. 2017a).

The neurobiological link between prolonged performance of a cognitive task and impairment of exercise performance might reside in the anterior cingulate cortex (ACC) (Marcora et al. 2009), an area of the brain's prefrontal cortex that is affected by MF (Lorist et al. 2005). Marcora et al. speculate that PCT affects the ACC's effort-based decision making by increasing perception of effort during the exercise bout (Marcora et al. 2009; Pageaux et al. 2014, 2015; Smith et al. 2015, 2016). Interestingly, when subject perception of effort during exercise is decreased hypnotically with work rate held constant, the reduction in RPE is associated with decreased cerebral blood flow in this area of the brain (Williamson et al. 2001). While it is important to note that there is no evidence to suggest a causal relationship between RPE and ACC activity, it is possible that an alteration in ACC activity could be an objectively measurable link between PCT and decreased exercise performance (Marcora et al. 2009).

The purpose of this study was to determine the influence of PCT on exercise performance in competitive athletes. We asked athletes involved in a variety of sports (ATH) and untrained participants (UNT) to perform 6 min of CWR exercise followed by a 6-min TT both with (PCT) and without (CON) completion of a 30-min PCT protocol that required response inhibition. We hypothesised that PCT would decrease the amount of work completed during TT in UNT, but not ATH. During both the PCT and the exercise bout comprising CWR and TT, we used near-infrared spectroscopy (NIRS) to assess cerebral oxygenation as a way to infer frontal-lobe brain activation (Ehlis et al. 2005; Sakatani

et al. 1999; Schroeter et al. 2002). We hypothesised that frontal-lobe activation would be greater in the PCT compared to control condition for UNT, but not ATH.

## Methods

### Participants

Ten competitive male athletes (mean ± SD: age 27.4 ± 6.3 year; stature 1.79 ± 0.06 m; body mass 75.6 ± 9.7 kg) and ten healthy males who did not participate in structured physical activity (age 25.8 ± 4.6 year; stature 1.83 ± 0.06 m; body mass 93.3 ± 17.0 kg) were recruited to participate in this study, which was approved by the University of Exeter Research Ethics Committee. The ATH group comprised three cyclists, two triathletes, two footballers, a distance runner, a crossfit athlete and a boxer. All participants were required to provide written informed consent, complete a medical-health questionnaire and provide an exercise log to assess training status prior to participating in the study. The total time spent exercising per week at low, moderate, high and very-high intensities was calculated for each participant and training status was quantified using a physical-activity rating tool (PA-R). The ATH group trained > 9.5 h per week and had a PA-R score of 9.7 (range 8–10), whereas the UNT group did < 3 h of physical activity per week and had a PA-R score of 3.8 (range 1–6). Participants were instructed to refrain from alcohol ingestion or strenuous exercise participation for 24 h prior to each laboratory visit and to arrive at the laboratory in a rested and fully hydrated state.

### Experimental overview

The participants reported to the laboratory on four occasions at the same time of day (± 2 h) with visits separated by ≥ 24 h. All participants completed all testing sessions in 2–4 weeks. During the first visit, participants completed a ramp incremental cycling test to the limit of tolerance to assess cardiorespiratory fitness. The second visit was used to familiarise participants with the procedures required for the two exercise trial sessions, which comprised visits three and four. At the beginning of these sessions, an intravenous cannula was inserted in the antecubital vein of the subject's arm to permit blood sampling throughout the exercise bout. The order in which these exercise sessions were performed was randomised. A schematic representation of the timeline of events in sessions 3 and 4 is provided in Fig. 1.

### Cognitive tasks

Both exercise trial sessions began with participants performing cognitive tasks designed either to perturb (PCT) or maintain (CON) a neutral mental state. The PCT comprised 30 min of performance of a computer-based modified version of the Stroop colour-word task and N-back task (E-Prime 2.0; Psychology Software Tools, Inc. 2013). The Stroop colour-word and N-back tasks were alternated on a 3- and 2-min loop, respectively. Participants were seated comfortably at a desk in the laboratory beside the cycle ergometer. The computer monitor was positioned at eye level and a keyboard with four colour-coded keys (red, green, blue and yellow) was placed in front of the participant who was wearing both ear plugs and noise-cancelling headphones to eliminate auditory distraction.

The Stroop colour-word task involved the presentation of text with stimuli classified as: (1) 'congruent' where a word describing a colour ('Red', 'Green', 'Blue' or 'Yellow') was presented with the text written in the same colour as the word; (2) 'incongruent' where the same words were presented, but the colour of the text did not match the word (e.g. the word 'Red' was written with blue font); or (3) neutral where 'XXX' written in one of the font colours used for the congruent and incongruent conditions was presented. Each text item was presented for 1700 ms followed by a blank screen for 700 ms with the stimuli balanced such that an equal number of congruent, incongruent and neutral items were presented. Subjects were instructed to push the key which indicated the colour in which the word was presented. The N-back task involved the display of a series of single letters presented on the screen one at a time for 1000 ms

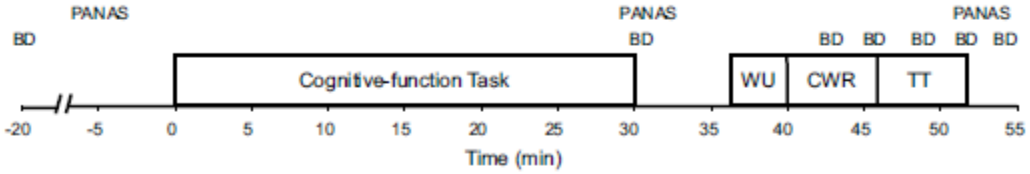


**Fig. 1** Schematic illustration of the experimental protocol during laboratory visits three and four. A 15-min exercise bout comprising a 3-min warm-up (WU), 6-min constant-work-rate bout at 70%Δ (CWR) and 6-min time trial (TT) was initiated 7 min following completion of a cognitive task that either perturbed (experimental condition) or maintained (control condition) a neutral mental state. The times at which blood was drawn for sampling (BD) and positive and negative affect were assessed (PANAS) are indicated

followed by a blank screen for 750 ms. If the letter on display was the same as the letter presented two letters previously (2-back protocol), subjects were instructed to press the green key. If the letter was different, subjects were instructed to press the blue key. Labels indicating 'yes' or 'no' were placed above the green and blue key, respectively. A one-minute practice session comprising 30 s of performance of each cognitive task was allowed prior to beginning the trial to ensure understanding. Subjects were told that they were being tested on both response accuracy and reaction time, which were each recorded and averaged for 5-min bins for the entire PCT (Fig. 1).

The CON condition consisted of watching Episode 5 of 'The Life of Birds' documentary by Sir David Attenborough for 30 min. The purpose was to establish a 'neutral mood' for the subject (Wirth et al. 2011). Participants were seated in the same chair at the same desk as during the PCT. Participants also wore the same noise-cancelling headphones; however, in this case, the soundtrack of the documentary was provided for auditory engagement.

## Exercise tasks

### Ramp incremental cycling test

On the first laboratory visit, participants completed a ramp incremental cycling test for determination of the gas exchange threshold metabolic rate ($GET_{met}$) and peak rates of oxygen uptake and work ($\dot{V}O_{2peak\text{-}INC}$ and $W_{peak\text{-}INC}$, respectively). This test was performed on an electronically braked cycle ergometer (Lode, Excalibur, Groningen, The Netherlands) with seat height and handles adjusted for comfort. The settings were recorded so that the setup could be replicated in subsequent tests. Participants began with 3 min of baseline cycling at 20 W after which the work rate was increased in a continuous manner at 25 (UNT) or 35 (ATH) W per min (i.e. 1 W per 2.4 or 1.7 s, respectively) until the limit of tolerance was reached. Participants were instructed to cycle at 80 rpm throughout with the test terminated when the pedal rate fell by > 5 rpm for more than 5 s despite strong verbal encouragement. Breath-by-breath pulmonary gas-exchange data were collected continuously throughout the test and averaged into 10-s bins. The $\dot{V}O_{2peak\text{-}INC}$ was defined as the highest 30-s rolling-average value during the test. The $GET_{met}$ was estimated using a cluster of measures including: (1) the first disproportionate increase in the rate of carbon dioxide output ($\dot{V}CO$) from visual inspection of individual plots of $\dot{V}CO_2$ versus $\dot{V}O_2$; (2) an increase in minute ventilation ($\dot{V}_E$) relative to $\dot{V}O_2$ with no increase in $\dot{V}_E$ relative to $\dot{V}CO_2$; and (3) an increase in end-tidal $O_2$ tension with no fall in end-tidal $CO_2$ tension. For each incremental test, $GET_{met}$ was estimated independently by experienced examiners and a consensus estimate was established. To align $GET_{met}$ with the work rate that precipitated it ($W_{GET}$), account was made for the mean response time of the $\dot{V}O_2$ response, which was estimated as 40 s (Whipp et al. 1981) (i.e. ~17 and ~23 W of work for UNT and ATH, respectively). The CWR bouts completed in subsequent laboratory sessions were completed with the work rate set at 70%Δ ($W_{GET}$ plus 70% of the difference between $W_{GET}$ and $W_{peak\text{-}INC}$).

### Constant-work-rate cycling test and cycling time trial

On the third and fourth laboratory visits, participants performed a CWR/TT cycling bout that was initiated 7 min following completion of the cognitive task. The CWR portion of the bout began with 3 min of warm-up cycling at 20 W. Following this baseline period, work rate was increased in a step fashion to 70%Δ (see above) after which participants cycled for 6 min. Participants were instructed to maintain cadence at 80 rpm during this bout. Immediately following completion of 6 min of CWR cycling, the ergometer mode was changed from hyperbolic to linear so that the subject could perform a 6-min TT during which they were instructed to complete as much work as possible. To ensure consistency across subjects, resistance on the pedals during the TT was set specifically so that they would attain their power output at 70%Δ when pedalling at 80 rpm (linear factor = power/cadence$^2$). To aid in TT performance, a clock was visible so that participants were aware of the time remaining. The same investigator was present at each testing session and similar verbal encouragement was provided every 30 s to ensure consistent motivation across experimental conditions. Power output was measured continuously throughout the TT and the total work completed ($Work_{TT}$) was calculated in kilojoules by multiplying the mean power output in watts by 360 s. Bin-averaged 30-s power outputs were also calculated for the 6-min TT.

## Physiological measures

### Metabolic data

During all exercise tests, pulmonary gas exchange and ventilation were measured breath by breath using an online gas analyzer (Jaeger Oxycon Pro, Hoechberg, Germany). Participants wore a nose clip and breathed through a low-dead-space, low-resistance mouthpiece and an impeller turbine assembly (Jaeger Triple V). A capillary line was connected to the mouthpiece and inspired and expired gas volume and gas concentration signals were sampled continuously at 100 Hz using paramagnetic ($O_2$) and infrared ($CO_2$) analyzers (Jaeger Oxycon Pro, Hoechberg, Germany). The gas analyzers were calibrated before each test with gases of known concentration and the turbine

volume transducer was calibrated using a 3-L syringe (Hans Rudolph, Kansas City, MO). The volume and concentration signals were time aligned by accounting for the delay in capillary gas transit and analyser rise time relative to the volume signal.

Total $O_2$ consumed was determined for the CWR bout by integrating the area under the $\dot{V}O_2$/time curve for 0–120 s, 0–180 s and the entire 360 s. Total $O_2$ consumed during the entire TT was determined in a similar manner. The peak $\dot{V}O_2$ during CWR ($\dot{V}O_{2peak-CWR}$) and TT ($\dot{V}O_{2peak-TT}$) were defined as the highest 10-s mean $\dot{V}O_2$ values from 0 to 360 s and 360 to 720 s of the CWR and TT bout.

### Heart rate

Subjects' HR was measured continuously during all exercise tests using short-range radiotelemetry (Polar S610; Polar Electro Oy, Kempele, Finland) with a sampling frequency of 5 s. The mean HR was calculated for the final 60 s of baseline cycling and for 0–120 s, 120–240 s and 240–360 s of CWR. The mean HR during TT was determined in a similar manner and the average HR during each 30-min cognitive task was also calculated.

### Cerebral oxygenation

The oxygenation of the frontal lobe was monitored using NIRS (NIRO 300; Hamamatsu Photonics KK, Hiugashiku, Japan). The NIRS probe consisted of a rubber holder containing a detector and an emitter separated by 4 cm. Double-sided tape was attached to the probe, which was adhered 3 cm above the left eyebrow (i.e. between Fp1 and F3 of the prefrontal cortex; PFC) according to the modified International EEG 10–20 system (Perrey 2008). The NIRS data were collected at a sampling frequency of 2 Hz and the Beer–Lambert law was used to calculate changes in the concentration of tissue oxyhemoglobin ($\Delta[HbO_2]$) and deoxyhemoglobin ($\Delta$[HHb]) using optical densities and a differential path length factor of 5.93 (Duncan et al. 1995). The $\Delta[HbO_2]$ and $\Delta$[HHb] were summed to provide an estimate of total blood volume ($\Delta[Hb_{tot}]$) and tissue oxygenation index (TOI) was also calculated. The NIRS measurement began with 60 s of resting data collection prior to the cognitive task and subsequent changes were calculated relative to this baseline. The TOI was expressed as a percentage. The $\Delta[HbO_2]$, $\Delta$[HHb], $\Delta[Hb_{tot}]$ and TOI data collected during the cognitive tasks and CWR/TT bouts were averaged into 300- and 120-s bins, respectively, and overall exercise values were also determined by averaging the data for the entire 360 s of both CWR and TT.

### Blood sampling

Venous blood samples were collected into lithium-heparin (LH) and ethylenediaminetetraacetic-acid (EDTA) vacutainers (Becton–Dickinson, New Jersey, USA) on seven occasions throughout laboratory sessions three and four (Fig. 1). Immediately after sampling, 200 µL of blood was extracted from the LH vacutainer into 200 µL of Triton-X-100 solution (Triton X-100, Amresco, Salon, OH) and plasma lactate and glucose concentrations were measured ([lactate] and [glucose], respectively) (YSI 1500; Yellow Springs Instrument, Yellow Springs, OH). The remaining blood was centrifuged at 4000 rpm for 8 min at 4 °C after which plasma was extracted and analyzed for potassium and sodium concentrations ($[K^+]$ and $[Na^+]$, respectively) (9180 Electrolyte Analyzer, F. Hoffmann-La Roche, Basel, Switzerland). The plasma obtained from the EDTA vacutainers was frozen at − 80 °C and, upon completion of the study, defrosted at room temperature and analysed for cortisol concentration ([cortisol]) using an ELISA kit (Abnova, Taiwan).

### Psychological measures

Subject affect was assessed using the positive and negative affect scale (PANAS; Watson et al. 1988). This was administered before and immediately following the cognitive tasks and 1 min after completion of TT (Fig. 1). Participants were instructed to answer the questions as they were feeling 'right now'. The questionnaire consisted of 20 words that describe positive ($n = 10$; e.g. 'excited', etc.) and negative ($n = 10$; e.g. 'irritable', etc.) feelings presented in random order. Participants were instructed to record a number between one and five (1 = very slight or not at all; 2 = a little; 3 = moderately; 4 = quite a bit; 5 = extremely) next to each word. The negative and positive scores were then summed separately. Total scores for positive and negative affect each ranged from 10 to 50.

## Statistical analysis

Statistical analysis was conducted using SPSS version 22.0 (SPSS Armonk, NY) and all data are reported as mean ± SD. Across-group comparisons for $\dot{V}O_{2peak\text{-}INC}$, $W_{peak\text{-}INC}$, $W_{GET}$, $GET_{met}$, power output at 70%Δ, PA-R and total exercise time per week in the various intensity zones were made using paired-sample $t$ tests. A 2 × 2 (condition × group) repeated-measures ANOVA (RMANOVA) was used to compare the mean HR response during the cognitive tasks and $W_{TT}$ while a 2 × 6 (group × time) RMANOVA was used to compare PCT response accuracy and reaction time. A 2 × 2 × 3 (condition × group × time) RMANOVA was employed to compare PANAS positive- and negative-affect measurements and the $\Delta[HbO_2]$, $[\Delta$HHb], $[\Delta Hb_{tot}]$ and TOI overall exercise

responses while a 2×2×6 RMANOVA was used to compare the 300- and 120-s mean responses for $\Delta[HbO_2]$, [ΔHHb], $[\Delta Hb_{tot}]$ and TOI during the cognitive tasks and CWR/TT, respectively. Total $O_2$ consumed during each 120 s of CWR/TT was also analysed using a 2×2×6 RMANOVA. Plasma [cortisol] measurements for pre cognitive task, post cognitive task, end-TT and 3-min-post end-TT were compared using a 2×2×4 RMANOVA and RMANOVA was also used to analyse plasma [lactate], [glucose], $[K^+]$ and $[Na^+]$ measurements at the same time points in addition to mid-CWR, end-CWR and mid-TT (2×2×7). In addition, a 2×2×7 RMANOVA was used to compare HR and $\dot{V}O_2$ measurements for the time bins averaged for baseline and throughout the exercise. Finally, 30-s mean power outputs during TT were analysed using a 2×2×12 RMANOVA. In all cases, when the sphericity assumption was violated, the Greenhouse–Geisser correction was employed and post-hoc tests were performed using pairwise comparison with Bonferroni correction. Statistical significance was accepted when $P<0.05$.

## Results

### Participant characteristics

Between-group comparisons for $\dot{V}O_{2peak\text{-}INC}$, $W_{peak\text{-}INC}$, $W_{GET}$, PA-R and total exercise time per week are provided in Table 1. The $\dot{V}O_{2peak\text{-}INC}$, $W_{peak\text{-}INC}$, $W_{GET}$ and PA-R were all significantly greater for ATH compared to UNT ($P<0.01$). Furthermore, compared to UNT, ATH performed a significantly greater amount of exercise per week at high and very-high intensities. The power output at 70%Δ that was maintained during CWR was greater for ATH compared to UNT (325 ± 34 v. 224 ± 24 W; $P<0.001$). Due to technical difficulties, data collected during the PCT for one member of the ATH group were excluded from these results.

**Table 1** Fitness characteristics for the athletes and untrained subjects who were assessed in this study

| | ATH | UNT |
|---|---|---|
| $\dot{V}O_{2peak\text{-}INC}$ (mL kg$^{-1}$ min$^{-1}$) | 58.3 ± 4.1 | 39.0 ± 7.3* |
| $W_{peak\text{-}INC}$ (W) | 401 ± 36 | 280 ± 27* |
| $W_{GET}$ (W) | 149 ± 32 | 91 ± 25* |
| PA-R | 9.7 ± 0.7 | 3.8 ± 2.2* |
| Total exercise time (h week$^{-1}$) | 9.5 ± 2.5 | 3.2 ± 1.9* |
| Low-intensity exercise time (h week$^{-1}$) | 0.5 ± 0.8 | 1.2 ± 2.2 |
| Moderate-intensity exercise time (h week$^{-1}$) | 2.3 ± 3.1 | 1.2 ± 1.8 |
| High-intensity exercise time (h week$^{-1}$) | 3.8 ± 1.4 | 0.8 ± 0.9* |
| Very-high-intensity exercise time (h week$^{-1}$) | 2.9 ± 1.7 | 0.0 ± 0.0* |

Values are presented as mean ± SD

$\dot{V}O_{2peak\text{-}INC}$ peak rate of oxygen uptake during incremental test, $W_{peak\text{-}INC}$ peak rate of work during incremental test, $W_{GET}$ estimated rate of work at gas exchange threshold, *PA-R* physical-activity rating

*Significantly different from ATH ($P<0.01$)

### Psychological responses during cognitive tasks

The results of the PANAS assessment before and after the cognitive tasks are depicted in Fig. 2. For positive affect, UNT had a significantly lower value following PCT compared to CON (19.9 ± 7.5 v. 24.3 ± 4.6; $P=0.036$) while for negative affect, ATH had a significantly higher value following PCT compared to CON (15.1 ± 3.7 v. 12.2 ± 2.7; $P=0.029$).

### Physiological responses during cognitive tasks

Compared to ATH, UNT had a higher mean HR during both cognitive tasks (PCT, 75 ± 9 v. 59 ± 6 b min$^{-1}$; CON, 69 ± 7 v. 58 ± 6 b min$^{-1}$; $P<0.005$ in both cases). A between-condition difference was also present for UNT who demonstrated a higher HR during PCT compared to CON ($P=0.002$). Conversely, there was no difference in mean HR between PCT and CON for ATH. There were no between-group or between-condition differences in plasma [lactate], [glucose], $[K^+]$, $[Na^+]$ or [cortisol] before or after the cognitive tasks. There were also no changes in any of the blood variables during the cognitive tasks except for $[K^+]$, which increased slightly for UNT during PCT (post 4.4 ± 0.5 mM; pre 3.9 ± 0.30 mM; $P=0.002$).

### Cerebral-oxygenation responses during cognitive tasks

There were no between-group differences for cerebral $\Delta[HbO_2]$, Δ[HHb] or $\Delta[Hb_{tot}]$ at any time point during either cognitive task; however, UNT had a higher TOI at all time points during both PCT and CON. Furthermore, for UNT, TOI was greater than baseline for all time points beyond 10 min during both cognitive tasks, whereas for ATH, TOI did not change. There were no between-condition differences for any of the cerebral-oxygenation variables for either group at any time point during the cognitive tasks.

### Performance during prolonged cognitive task

Response accuracy and reaction time did not change significantly over time during the PCT for either group. Furthermore, there were no between-group differences for response accuracy at any time point during the 30-min PCT task; however, UNT had a significantly shorter response time compared to ATH at all time points other than the final 5 min.

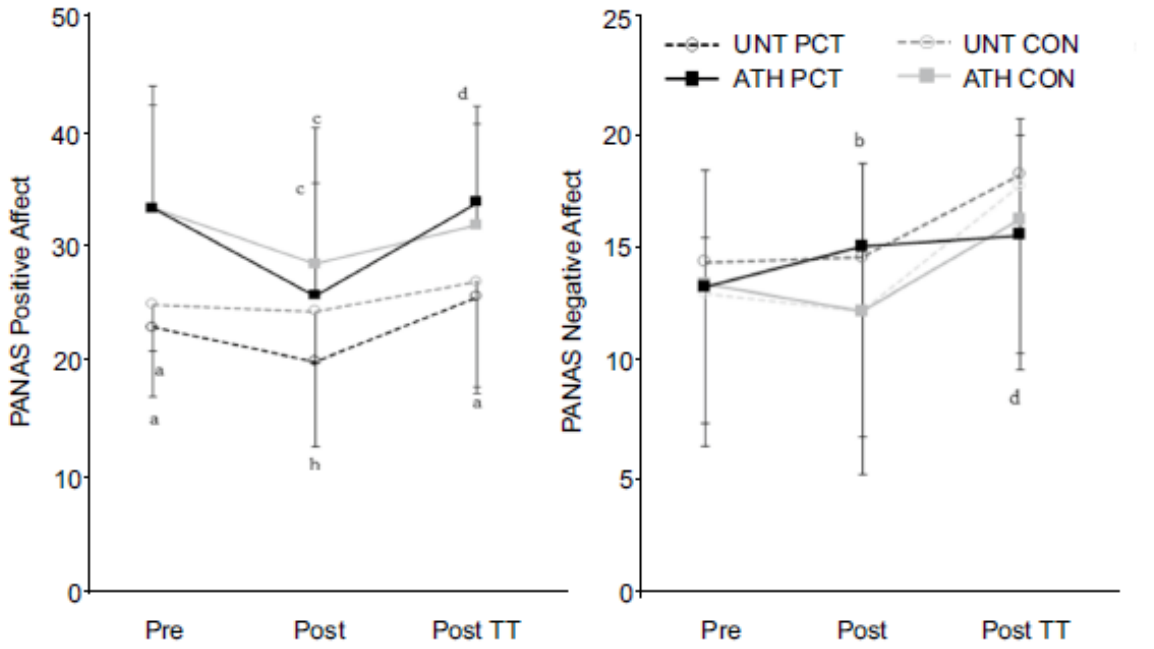


**Fig. 2** Positive (left panel) and negative (right panel) affect ratings for competitive athletes (ATH) and untrained participants (UNT) pre and post task that either perturbed (PCT) or maintained (CON) a neutral mental state. Post time trial (TT) values are also provided for comparison. Values are mean ± SD. **a** Significant difference compared to ATH group ($P < 0.05$); **b** significant difference compared to CON condition ($P < 0.05$); **c** significant difference compared to baseline value ($P < 0.05$); **d** significant difference compared to post-cognitive-task value ($P < 0.05$)

### Psychological responses during exercise

The results of the PANAS assessment following the TTs are shown in Fig. 2. Compared to post-cognitive task, post-exercise positive affect was significantly increased for ATH ($P = 0.033$) whereas post-exercise negative affect was significantly increased for UNT ($P = 0.012$). No between-condition differences were present for post-exercise positive or negative affect for either group.

### Physiological responses during exercise

Group-by-condition comparisons for $\dot{V}O_2$ variables and total $O_2$ consumed during the CWR/TT bout under PCT and CON conditions are shown in Table 2. There were no significant differences between PCT and CON for any metabolic variables for ATH, whereas for UNT, $\dot{V}O_{2peak\text{-}TT}$ was lower in PCT compared to CON ($P = 0.04$). There were no between-condition differences for $\dot{V}O_2$ or HR during any of the time bins averaged during CWR or TT.

Group-by-condition comparisons for plasma [lactate], [glucose] and [cortisol] at baseline, end-TT and 3-min-post end-TT are provided in Table 3. Plasma [lactate] increased from baseline in a similar manner during exercise for both groups during both conditions, whereas there was no change in plasma [cortisol] from baseline during exercise for either group during either condition. However, 3 min following the exercise bout, plasma [cortisol] was significantly greater compared to immediately post-exercise for UNT regardless of the cognitive task, whereas for ATH, this was only the case for CON. There were no other temporal changes or between-condition differences for any of the blood-sampling variables during or post-exercise except for $[K^+]$ and $[Na^+]$, which each increased from baseline in a similar manner during exercise for both groups during both conditions.

### Cerebral-oxygenation responses during exercise

The mean ± SD values for cerebral-oxygenation responses during CWT/TT are depicted for each group in Fig. 3. For ATH, there were increases in the $\Delta[HbO_2]$, $\Delta[HHb]$ and $\Delta[Hb_{tot}]$ responses from CWR to TT in both PCT and CON ($P < 0.001$). Increases in $\Delta[HbO_2]$ and $\Delta[Hb_{tot}]$ were also present in the transition from CWR to TT for UNT in PCT and CON ($P < 0.001$), but in UNT the $\Delta[HHb]$ decreased significantly during CWR and increased during the TT in the PCT condition ($P < 0.05$). There was a between-group difference for TOI with UNT having a lower overall exercise

**Table 2** Metabolic data for athletes (ATH) and untrained subjects (UNT) during exercise performed after cognitive tasks that perturbed (PCT) or maintained (CON) a neutral mental state

| | ATH | | UNT | |
|---|---|---|---|---|
| | PCT | CON | PCT | CON |
| $\dot{V}O_{2base}$ (L min$^{-1}$) | 1.13±0.11 | 1.07±0.23 | 1.14±0.10 | 1.17±0.13 |
| $\dot{V}O_{2peak\text{-}CWR}$ (L min$^{-1}$) | 4.48±0.45 | 4.46±0.41 | 3.35±0.33* | 3.44±0.35* |
| $\dot{V}O_{2peak\text{-}TT}$ (L min$^{-1}$) | 4.50±0.46 | 4.49±0.50 | 3.55±0.29* | 3.64±0.32*† |
| Total $O_2$ consumed 0–120 s (L) | 4.9±0.4 | 4.9±0.4 | 3.4±0.5* | 3.4±0.6* |
| Total $O_2$ consumed 0–180 s (L) | 8.8±0.7 | 8. 8±0.7 | 6.1±0.9* | 6.3±0.9* |
| Total $O_2$ consumed 0–360 s (L) | 21.6±1.6 | 21.5±1.7 | 15.6±2.1* | 15.9±2.1* |
| Total $O_2$ consumed TT (L) | 25.2±2.2 | 25.1±2.0 | 19.2±2.4* | 19.6±2.5* |

Values are presented as mean±SD

$\dot{V}O_{2base}$ rate of oxygen uptake during baseline cycling, $\dot{V}O_{2peak-CWR}$ peak rate of oxygen uptake during the constant-work-rate bout, $\dot{V}O_{2peak-TT}$ peak rate of oxygen uptake during the time trial

*Significantly different from ATH within condition ($P<0.05$)

†Significantly different from PCT within group ($P<0.05$)

**Table 3** Blood-sampling data for athletes (ATH) and untrained subjects (UNT) before and following exercise performed after cognitive tasks that perturbed (PCT) or maintained (CON) a neutral mental state

| | ATH | | UNT | |
|---|---|---|---|---|
| | PCT | CON | PCT | CON |
| Baseline [lactate] (mM) | 0.8±0.2 | 1.0±0.3 | 1.2±0.5 | 1.0±0.5 |
| End-exercise [lactate] (mM) | 10.7±2.1 | 10.2±1.7 | 8.8±1.9 | 8.6±1.7 |
| Three-min post [lactate] (mM) | 10.7±2.0 | 8.9±2.8† | 9.6±1.8 | 9.7±2.3 |
| Baseline [glucose] (mM) | 4.2±0.5 | 4.8±1.3 | 4.6±1.2 | 4.5±0.8 |
| End-exercise [glucose] (mM) | 4.5±0.7 | 4.6±0.7 | 4.1±1.0 | 3.8±0.9 |
| Three-min post [glucose] (mM) | 5.7±1.0 | 5.0±1.5 | 5.2±1.8 | 4.6±1.0 |
| Baseline [cortisol] (μg dL$^{-1}$) | 11.5±6.2 | 10.4±5.4 | 12.5±4.3 | 12.4±2.8 |
| End-cognitive-task [cortisol] (μg dL$^{-1}$) | 10.3±3.4 | 8.8±2.8 | 10.0±2.1 | 9.9±2.3 |
| End-exercise [cortisol] (μg dL$^{-1}$) | 10.0±3.5 | 8.6±2.5 | 10.4±2.7 | 10.5±2.5 |
| Three-min post [cortisol] (μg dL$^{-1}$) | 10.6±3.2 | 10.0±2.4 | 12.1±2.6 | 12.8±2.7* |

Values are presented as mean±SD

*Significantly different from ATH within condition ($P<0.05$)

†Significantly different from PCT within group ($P<0.05$)

response compared to ATH (63±9 v. 71±8%, $P<0.05$). Moreover, the overall $\Delta[HbO_2]$ response during exercise was greater during PCT compared to CON for ATH but greater during CON compared to PCT for UNT ($P=0.025$ and 0.028, respectively). Specifically, ATH had a greater $\Delta[HbO_2]$ for the PCT condition during both CWR and TT, whereas UNT had a reduced response with PCT during TT. ATH also had a greater $\Delta[Hb_{tot}]$ for PCT compared to CON during both CWR and TT whereas there was no between-condition difference for UNT during either form of exercise.

### Performance during the TT

The 30-s mean power outputs throughout TT are depicted in Fig. 4 (top panels). Power output increased significantly during TT for ATH and UNT; however, there were no between-condition differences in power output at any time point during TT in either group. There were also no between-condition differences for work completed during TT for either group (Fig. 4, bottom panels).

## Discussion

The main original finding of this investigation was that highly-trained competitive athletes did not experience a decline in self-paced TT performance when exercise was performed shortly after a prolonged challenging cognitive task. This confirms the initial part of our first hypothesis and is consistent with the notion that trained competitive athletes possess characteristics that are resilient to the potential/putative adverse effect of PCT on exercise performance. However, contrary to the second part of our first hypothesis, the exercise performance of UNT was not adversely affected by PCT. Furthermore, with respect to cortical activation, NIRS measurements suggested a greater frontal-lobe activity

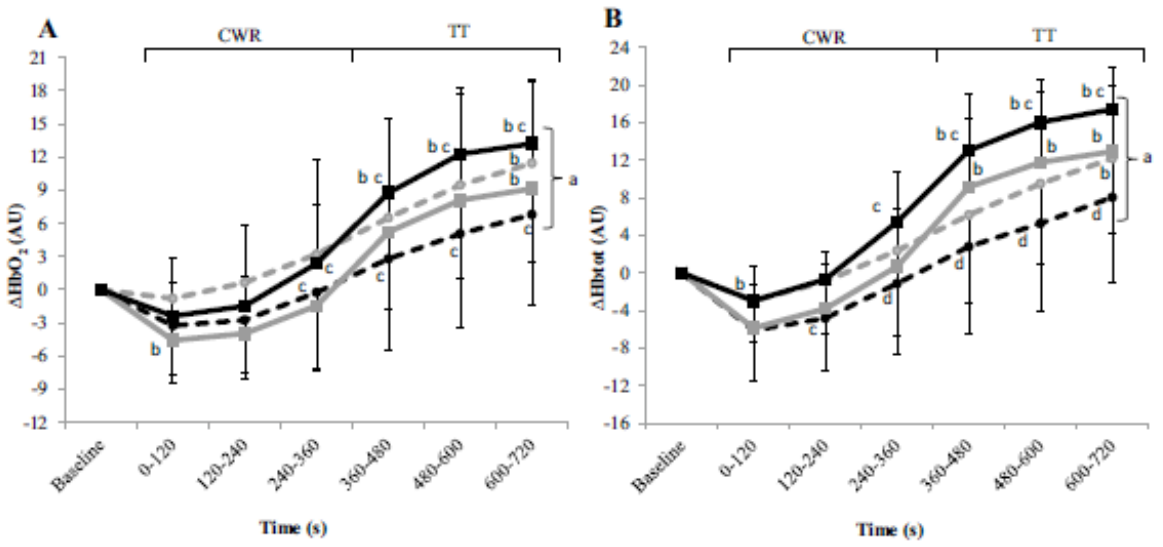


**Fig. 3** Cerebral $\Delta HbO_2$ (left panel) and $\Delta HbO_{tot}$ (right panel) for competitive athletes (squares) and untrained subjects (circles) prior to and during a 12-min bout of constant-work-rate (CWR) and time-trial (TT) cycling following cognitive-function tasks that either perturbed (black) or maintained (gray) a neutral mental state. **a** Significant main effect of time ($P<0.001$). **b** Significantly different from baseline value ($P<0.05$). **c** Significantly different from CON ($P<0.05$). **d** Significantly different from ATH ($P<0.05$)

for ATH during exercise after PCT, which is in contrast to our second hypothesis. Collectively, these findings indicate complexity in the possible ergolytic effects of PCT during self-regulated exercise.

In the present study, we chose to assess the influence of PCT on exercise performance in two distinctly different groups of individuals based on their experience with competitive sport and exercise training. Our objective was to determine if athletic status influences the degree to which PCT may impair exercise performance. Most previous investigations have involved assessment of a single group of participants with similar fitness characteristics (for overview, see Table 5 by Van Cutsem et al. 2017b; cf. Martin et al. 2016). Indeed, most of the research that has identified an ergolytic effect of PCT has involved individuals who performed regular physical training, but not competitive sport (Marcora et al. 2009; Brownsberger et al. 2013; Pageaux et al. 2013, 2014; MacMahon et al. 2014). In investigations that have assessed the influence of PCT in competitive athletes, results have been mixed (Duncan et al. 2015Martin et al. 2015; Martin et al. 2016; Smith et al. 2016; Van Cutsem et al. 2017a). In the only study on professional endurance athletes, Martin et al. (2016) reported that PCT did not alter perception of effort or performance of road cyclists during a 20-min TT. However, an ergolytic effect of PCT was observed for recreational cyclists in that study (Martin et al. 2016) with other studies confirming such an effect for soccer players (Smith et al. 2016) and team-sport athletes (Smith et al. 2015) during intermittent running. In other studies, PCT did not affect 'anaerobic' performance in team-sport players (Duncan et al. 2015) or mean power output during the final 30 s of all-out cycling for either team-sport players or triathletes (Martin et al. 2015). With respect to the latter finding, this 'end-test power' approximates the critical power (CP; Vanhatalo et al. 2007), a parameter that reflects the greatest metabolic rate that can be sustained without progressive increase in muscle metabolic perturbation (Poole et al. 2016). Moreover, work completed above end-test power during the all-out bout was also unaffected by PCT (Martin et al. 2015). As these two parameters collectively dictate time to exhaustion during high-intensity exercise (Poole et al. 2016), this implies that high-intensity endurance performance (e.g. CWR time to task failure) would not have been altered had it been assessed. Finally, PCT did not impair cycling TT performance for cyclists/triathletes in the heat (Van Cutsem et al. 2017a). While these disparate findings might reflect differences in the cognitive challenge used to perturb mental state and/or different characteristics of the criterion exercise challenge (e.g. CWR exercise or TT, continuous or intermittent, intensity and/or duration) our results appear to cohere with those of Martin et al. (2016) for professional athletes and suggest that competitive athletes do not experience an ergolytic effect of PCT during endurance-exercise performance.

One factor that should be considered when interpreting the present findings is that the 6-min cycling TT we chose

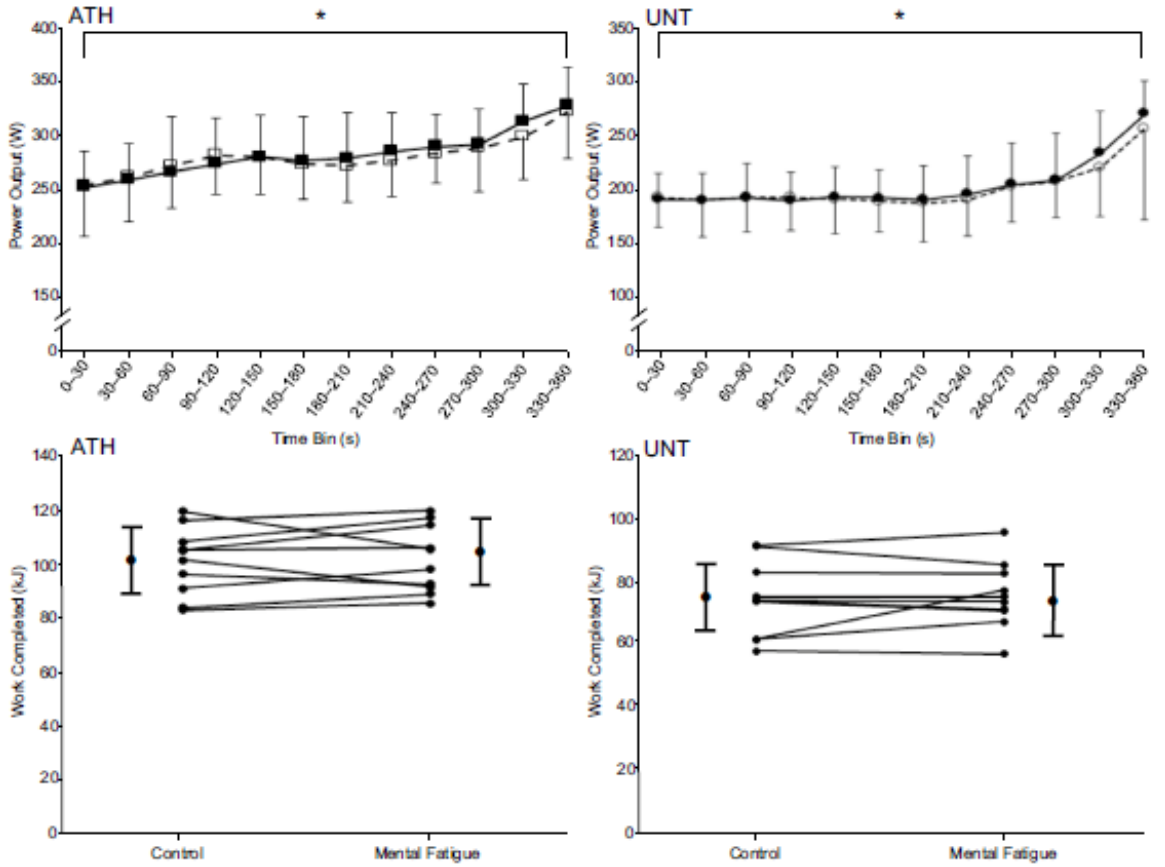


**Fig. 4** Thirty-second average power outputs for competitive athletes (left top panel) and untrained participants (right top panel) throughout the 6-min time trial that followed a cognitive-function task that either perturbed (closed squares/circles) or maintained (open squares/circles) a neutral mental state. Values are mean ± SD. *Main effect of time ($P < 0.05$). Notice how the self-regulated pacing profile was not altered by PCT in either group. Furthermore, as illustrated by the individual-subject and group mean ± SD data depicted in the lower panels, the work completed during the time trial for the competitive athletes (left; $n = 10$) and untrained participants (right; $n = 10$) was also unaffected by PCT

for performance assessment was different from those that have been used in the past for PCT research. For example, MacMahon et al. (2014) had participants perform a 3-km running TT that required ~ 12 min to complete, Pageaux et al. (2014) had participants perform a 5-km running TT, and Martin et al. (2016) had participants run for a constant duration of 20 min. In addition to employing a different exercise modality, we opted for a shorter TT to improve reliability (Currell and Jeukendrup 2008); however, the duration we chose should still be long enough to result in a high proportional contribution of oxidative contribution to total energy turnover. This is important because PCT appears to exert no effect on 'anaerobic' performance (Duncan et al. 2015).

In the present study, we also studied untrained individuals to provide a contrast with respect to the athletic and training experience of the participant. We reasoned that UNT would provide the ideal control group against which to compare the influence of PCT in ATH because they would presumably be most susceptible to a potential adverse effect on TT performance. However, contrary to our hypothesis, UNT also showed no reduction in work performed or change in pacing profile when exercising following PCT. It is important to note that inclusion of UNT qualifies ours as the first study to investigate the effect of PCT on exercise performance in participants with an 'average' cardiorespiratory fitness level (e.g. $\dot{V}O_{2peak}$, ~ 40 mL kg$^{-1}$ min$^{-1}$) and limited training experience. This raises the intriguing possibility that the psychological stress associated with unfamiliar intense exercise might have served as a cognitive stimulus for UNT that was mechanistically similar to (and, therefore, with respect to ergolytic effect, not additive with) that which was elicited by PCT. In this regard, it is interesting

to note that our two groups demonstrated markedly different patterns of two-dimensional affect response during the exercise challenge. Specifically, for ATH experienced with training, positive mood increased during exercise, whereas for UNT, it did not. This coheres with previous reports that trained individuals respond significantly more in the positive dimension during moderate- and high-intensity exercise compared to their untrained counterparts (Boutcher et al. 1997). Moreover, previous research confirms that negative affect also discriminates training status with untrained individuals demonstrating a decrease during exercise (primarily due to reductions in the 'afraid', 'jittery', 'nervous' and 'scared' categories) while trained individuals experience an increase during high-intensity exercise (Boutcher et al. 1997). A likely explanation is that unlike ATH who were familiar with intense exercise, UNT experienced a higher initial stress level that serves as a distraction which makes it difficult for them to focus on the 'normal' negative cognitive stimuli that are present during intense exercise (Pennebaker and Lightner 1980). The fact that PCT increased negative affect for the ATH, but not UNT, group in the present study is consistent with this possibility.

Being that the ergolytic effect of PCT has been attributed to MF (Marcora et al. 2009), it is possible that the lack of performance impairment in either group in the present study indicates that the prolonged cognitive tasks we employed did not elicit sufficient MF must be considered. We found that the reaction time and response accuracy were unaltered during the prolonged cognitive task, which involved a computer-based modified version of the incongruent Stroop colour-word task and N-back task for a continuous 30-min block. The incongruent Stroop, a cognitive task that requires inhibition of automatic responses to visual stimuli, has been used previously to induce MF prior to exercise and/or impair subsequent exercise performance. For example, despite the fact that MF was not directly measured in their study, Pageaux et al. (2014) found a reduction in self-paced 5-km TT running speed along with an increased perception of effort after 30 min of modified Stroop performance. Thirty minutes of Stroop performance also resulted in a reduction in running distance during the Yo–Yo Intermittent Recovery Test (Smith et al. 2016) along with an increased perception of effort during both Yo–Yo (Smith et al. 2016) and CWR-exercise performance (Pageaux et al. 2015). Finally, Martin et al. (2016) used the 30-min Stroop protocol when they found that athletic status determines the influence of MF on 20-min TT performance. Unlike these previous studies, however, we added the performance of the N-back task intermittently during the Stroop to reduce singular-task disengagement thereby decreasing the likelihood of a reduction in MF-inducing stimulus during the latter stages of the test (Tanaka et al. 2015). Nevertheless, it is important to recognise that the 30-min cognitive challenge that we employed might not have been long enough to achieve the psychobiologic perturbation necessary to impair subsequent exercise performance. Consequently, future research should be designed to explore the effect of longer PCTs on exercise performance in competitive athletes and untrained individuals.

In the present study, we observed a lower peak $\dot{V}O_2$ response during TT for the PCT compared to CON condition in UNT and a higher 3-min post [lactate] for the PCT compared to CON condition in ATH. However, despite these changes, which are consistent with the contention that PCT altered the metabolic challenge associated with performing the TT, exercise performance was not adversely affected in either group. Interestingly, we observed a greater HR response for untrained participants during PCT compared to the neutral-state documentary, which agrees with previous research (Pageaux et al. 2014, 2015). UNT also demonstrated a reduction in positive affect after performing the PCT. Conversely, for ATH, no difference in HR between PCT and control was observed, which likely reflects the fact that ATH show reduced autonomic and psychological stress responses compared to UNT (Rimmele et al. 2007). However, positive affect was decreased and negative affect was increased for ATH following PCT, which suggests that the intervention also alters mental state compared to the control condition in this type of individual.

During exercise, between-condition differences in $\Delta[HbO_2]$ were consistent with the contention that UNT possess a markedly different 'mind set' in response to an intense exercise challenge compared to ATH. Although not without potential limitations (e.g. signal contamination due to a thermoregulatory increase in skin blood flow to the forehead; Miyazawa et al. 2013), NIRS measurements of cerebral oxygenation during exercise are highly reliable (Bhambhani et al. 2006); hence, the absence of the between-condition difference in UNT supports an interpretation that the psychological stress of unfamiliar intense exercise presents an 'MF-like' cognitive stimulus that PCT does not exacerbate (see above). Interestingly, the presence of these stress-related changes in cortical oxygenation in ATH might provide insight into the resistance to MF displayed by professional cyclists in the study of Martin et al. (2016). Specifically, in that study, the professional athletes improved performance throughout the 30-min Stroop and performed a 20-min TT subsequently with unchanged RPE. This implies that the athletes did not experience MF despite the demanding nature of the PCT. Conversely, in our study, ATH experienced no change in performance during the PCT. The changes in cortical activation demonstrated by ATH during exercise in our study suggest that their mental state was altered by PCT, but that this did not adversely affect exercise performance.

Unlike previous studies investigating the effect of PCT on subsequent exercise performance, we chose not to measure

RPE in our study because questioning per se during intense exercise disrupts the focus of trained individuals thereby providing a stimulus that may also adversely influence cognitive state (increased negative affect due to increases in 'hostility' and 'irritability'; Boutcher et al. 1997). Moreover, we used a self-regulated TT as a way to assess exercise performance with and without perturbation of the neutral mental state. TT has been employed previously in some studies investigating the effect of PCT during exercise (Pageaux et al. 2014; MacMahon et al. 2014; Martin et al. 2016) whereas others have involved CWR bouts performed until task failure (Marcora et al. 2009; Pageaux et al. 2013). We chose TTs because of their validity with respect to performance in the athletic setting and greater reliability compared to CWR tests (Currell and Jeukendrup 2008). However, we also included a 6-min severe-intensity CWR bout immediately prior to the TT so that a variety of physiological measurements could be made during an exercise task that presented the same relative challenge to all participants (i.e. sustained constant work at a work rate determined relative to each participant's GET and peak work rates). We found that PCT did not alter the $\dot{V}O_2$, HR or blood [lactate] responses in either group during CWR, which is consistent with previous findings regarding the lack of influence of PCT on physiological factors during exercise (Marcora et al. 2009; Pageaux et al. 2015). Self-paced TT performance should be particularly sensitive to interventions that alter cognitive state. Specifically, the continuous decision-making that is present while establishing a pacing strategy likely involves significant PFC activation (Krawczyk 2002). Consistent with previous research (Pageaux et al. 2014; MacMahon et al. 2014), we found that PCT did not alter pacing strategy in either group. Furthermore, we found that both $\Delta[HbO_2]$ and $\Delta[Hb_{tot}]$ increased markedly from CWR to TT in both groups despite the fact that power output was lower during all but the final portion of the TT (i.e. a negative pacing strategy was employed). This implies that decisions regarding pacing were being established according to physiological/biomechanical factors and/or knowledge of the known endpoint of exercise rather than PFC activation per se (Billaut et al. 2010). Indeed, our NIRS data suggest that cerebral oxygenation was maintained over the course of both the severe-intensity CWR bout and TT. This coheres with the findings of Billaut et al. (2010) and is consistent with the contention that cerebral oxygenation is well preserved during severe-intensity exercise. Hence, PFC deoxygenation does not appear to hinder high-intensity exercise performance (Billaut et al. 2010; present study).

In summary, consistent with prior research on professional cyclists, we found that non-professional highly-trained individuals with a history of competition in a variety of sports do not experience an ergolytic effect of PCT at least during the performance of a 6-min cycling TT. However, a novel finding from the present study was that the TT performances of untrained participants were also not adversely impacted by a pre-exercise prolonged cognitive challenge. Although quantitatively similar responses were present in both groups, we speculate different mechanistic bases; specifically, the ability to maintain performance despite PCT in competitive athletes who are experienced with intense training and a psychobiological stress response to unfamiliar intense exercise that is similar to PCT for untrained individuals.

**Author contributions** IEC, AMJ, AV, JF, MIJ and SJB contributed to the conception and design of the experiment. IEC, RPG, STJM contributed to the collection, analysis and interpretation of the data. FJD, IEC AMJ, AV contributed to the writing of this article. All authors contributed to the critical revision of this manuscript and approved the final version.



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## Summary of chapter 4

Originally, the aim of the current thesis was to investigate how psychological (in form of mental fatigue) and physiological (in form of endurance exercise) fatigue might affect the power-duration relationship. As chapter 4 did not show an ergolytic effect of a prolonged cognitive task on severe-intensity exercise performance it was reasonable to expect that mental fatigue would not alter the power-duration relationship for severe-intensity exercise. The subsequent studies of the present thesis, therefore, did not address mental fatigue further and instead focused on the effects of prolonged, fatiguing endurance exercise on the power-duration relationship.

## Chapter 5. Effects of two hours of heavy-intensity exercise on the power-duration relationship

# Effects of Two Hours of Heavy-Intensity Exercise on the Power–Duration Relationship

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**ABSTRACT**

CLARK, I. E., A. VANHATALO, S. J. BAILEY, L. J. WYLIE, B. S. KIRBY, B. W. WILKINS, and A. M. JONES. Effects of Two Hours of Heavy-Intensity Exercise on the Power–Duration Relationship. *Med. Sci. Sports Exerc.*, Vol. 50, No. 8, pp. 1658–1668, 2018. **Introduction:** Changes in the parameters of the power–time relationship (critical power (CP) and $W'$) *during* endurance exercise would have important implications for performance. We tested the hypotheses that CP and $W'$, estimated using the end-test power (EP) and the work done above EP (WEP), respectively, during a the 3-min all-out test (3MT), can be reliably determined, and would be lower, after completing 2 h of heavy-intensity exercise. **Methods:** In study 1, six cyclists completed a 3MT immediately after 2 h of heavy-intensity exercise on two occasions to establish the reliability of EP and WEP. In study 2, nine cyclists completed a control 3MT, and a fatigued 3MT and constant power output tests to 30 min or the limit of tolerance ($T_{lim}$) below and above F-EP after 2 h of heavy-intensity exercise. **Results:** In study 1, EP (273 ± 52 vs 276 ± 58 W) and WEP (12.4 ± 4.3 vs 12.8 ± 4.3 kJ) after 2 h of heavy-intensity exercise were not different ($P > 0.05$) and were highly correlated ($r = 0.99$; $P < 0.001$). In study 2, both EP (F-EP: 282 ± 52 vs C-EP: 306 ± 56 W; $P < 0.01$) and WEP (F-WEP: 14.7 ± 4.9 vs C-WEP: 18.3 ± 4.1 kJ; $P < 0.05$) were lower after 2-h heavy-intensity exercise. However, maximum $O_2$ uptake was not achieved during exercise >F-EP and $T_{lim}$ was shorter than 30 min during exercise <F-EP (18.2 ± 10.7 min). **Conclusions:** The EP and WEP may be reliably determined after 2-h heavy-intensity exercise. The 8% and 20% reductions in EP and WEP, respectively, have important implications for performance during endurance exercise. The physiological characterization of EP (and, by extension, CP) may differ in a fatigued compared with a rested state. **Key Words:** ENDURANCE, FATIGUE, METABOLISM, PERFORMANCE, CRITICAL POWER, EFFICIENCY, $\dot{V}O_2$ PEAK

It is well established that the parameters of the hyperbolic power–time relationship, critical power (CP) and the work performed above CP ($W'$), are important parameters of fitness and strong predictors of endurance exercise performance (1–3). Critical power represents the boundary separating the "heavy" and "severe" exercise intensity domains. During exercise performed within the severe-intensity domain (>CP), the development of an oxygen uptake ($\dot{V}O_2$) "slow component" leads to the attainment of the maximal oxygen uptake ($\dot{V}O_{2max}$) with exhaustion occurring shortly thereafter (4). Moreover, compared with heavy-intensity exercise (<CP), severe-intensity exercise is accompanied by more pronounced reductions in muscle [phosphocreatine] ([PCr]) and pH, increases in muscle [lactate] and [inorganic phosphate], and neuromuscular dysfunction (5–8). The ability to sustain a specific submaximal power output during long duration events (i.e., >1–2 h) will be restricted by CP because this physiological threshold delineates power outputs that cannot be sustained for an appreciable duration from those that can (5–8). Accordingly, CP has important implications for pacing strategy and performance during longer duration athletic events (1,3). This might account for the observation that the mean speed sustained by elite distance runners during a marathon race lies within the heavy-intensity domain, at approximately 96% of critical speed (CS; the running equivalent to CP; 9).

The 3-min all-out cycle test (3MT) provides valid and reliable estimates of CP and $W'$, with the mean power output measured over the last 30-s of the test (defined as the end-test power [EP]) reflecting CP and the work completed above EP (WEP) reflecting $W'$ (9,10). These parameters are not affected when the 3MT is immediately preceded by 6 min of heavy-intensity exercise, whereas the EP is unchanged and WEP is reduced when the 3MT is immediately preceded by 2 to 4 min of severe-intensity exercise (11). Whether EP and WEP are impacted by longer duration heavy-intensity exercise is not known. As the duration of submaximal exercise is extended, muscle glycogen declines (5,12), particularly in type I muscle fibers (12,13), and the proportional contribution of central fatigue increases (14), factors which could potentially alter

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CP and/or $W'$ (15–17). To our knowledge, no study has investigated the effects of an extended period of submaximal exercise on CP and $W'$. The 3MT is well suited for this purpose because it removes the necessity for multiple prediction trials and expedites the estimation of CP and $W'$ (10).

Although the 3MT provides a valid and reliable estimate of CP and $W'$ when participants complete the test in a rested condition (i.e., without having undertaken prior fatiguing exercise; 8–10), it is not known whether EP and WEP can be reliably assessed during a 3MT completed after an extended bout of heavy-intensity exercise, which is likely to induce fatigue, and whether these parameters retain the same physiological meaning (i.e., equivalence to CP and $W'$). Specifically, it is not known whether EP measured after prolonged heavy-intensity exercise continues to discriminate power outputs wherein a physiological steady-state cannot be attained, and that result in the attainment of $\dot{V}O_{2max}$ and exhaustion within approximately 3 to 20 min, from power outputs wherein steady-state values of $\dot{V}O_2$ and blood [lactate] can be attained and exercise can be sustained for at least 20 to 30 min. Resolving whether EP is lowered and retains the same physiological significance in a fatigued state is of potential practical importance. If CP is lowered as long-duration endurance exercise is continued, this could compromise exercise efficiency and exacerbate muscle metabolic perturbation and neuromuscular dysfunction, compromising the ability to sustain a given race pace and ultimately limiting performance. Moreover, evidence for changes in CP and/or $W'$ during long-duration exercise would question the practice of predicting performance in such events from parameters measured from tests performed in a rested state (18).

The purpose of this study was twofold: first, to assess the reliability of EP and WEP, as derived from the 3MT, after 2 h of heavy-intensity exercise; and second, to study the effects that fatigue, induced by 2 h of heavy-intensity exercise, has on EP and WEP. We hypothesized that, after 2 h of heavy-intensity exercise: 1) the 3MT would provide reliable EP and WEP values; 2) EP and WEP would be significantly lower compared with values derived from a 3MT completed with no prior exercise; and, 3) EP would continue to demarcate the heavy and severe exercise intensity domains, as defined by an inability to stabilize blood [lactate] and the development of a $\dot{V}O_2$ slow component leading to the attainment of $\dot{V}O_{2peak}$, during exercise above, but not below, EP.

## METHODS

This article reports the results of two experiments. The first experiment was conducted to test the reliability of the 3MT after 2 h of heavy-intensity exercise, and the second experiment was conducted to assess the effect of 2 h of heavy-intensity exercise on EP and WEP estimated via the 3MT and the physiological responses to exercise performed below and above the "fatigued" EP. Six male cyclists (mean ± SD: age = 31 ± 6 yr, height = 1.80 ± 0.84 m, body mass = 76.8 ± 6.7 kg, $\dot{V}O_{2max}$ = 56.9 ± 9.8 mL·kg$^{-1}$·min$^{-1}$) participated in the first experiment and 12 male cyclists (age = 29 ± 11 yr, height = 1.77 ± 0.85 m, body mass = 75.9 ± 7.1 kg, $\dot{V}O_{2max}$ = 58.1 ± 7.7 mL·kg$^{-1}$·min$^{-1}$) participated in the second experiment, nine of whom also completed exercise tests below and above the fatigued EP. The participants were all training to compete in cycling or triathlon races and were familiar with ≥2-h exercise bouts, but were not elite. The participants were instructed to arrive at the laboratory in a rested and hydrated state, ≥2 h postprandial, having avoided alcoholic drinks and strenuous exercise for 24 h and caffeine for at least 2 h. The participants were asked to maintain their habitual diet throughout the study. The experimental procedures were approved by the Institutional Research Ethics Committee and informed consent was obtained from each participant before testing. All exercise tests were separated by a minimum of 24 h but the tests in which the 3MT was completed after the 2-h heavy-intensity exercise bouts (see below for more details) were separated by at least 48 h.

All the exercise tests in the first and second experiments were conducted using the same electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). During all tests, except the 3MT, the participants cycled at a self-selected pedal rate (70–90 rpm). The ergometer seat and handlebar configuration were adjusted for comfort during the first visit and were recorded and replicated for subsequent visits.

**Experiment 1: Reliability of 3MT parameters after 2-h heavy-intensity exercise.** Participants in the first experiment reported to the laboratory on five occasions over a period of approximately 3 wk. During the first visit, participants completed a ramp incremental exercise test for determination of $\dot{V}O_{2peak}$ and gas exchange threshold. Initially, each participant completed a 3-min baseline of pedalling at 20 W after which the power output was increased by 30 W·min$^{-1}$ until the participant was unable to continue. The limit of tolerance was determined when cadence fell >10 rpm below the target cadence for more than 5-s despite strong verbal encouragement. $\dot{V}O_{2peak}$ was determined as the highest 30-s mean value recorded during the test. The gas exchange threshold was defined as: 1) the first disproportionate increase in carbon dioxide output ($\dot{V}CO_2$) relative to $\dot{V}O_2$; and 2) an increase in the ventilatory equivalent for $O_2$ ($\dot{V}_E/\dot{V}O_2$) with no increase in the ventilatory equivalent for $CO_2$ ($\dot{V}_E/\dot{V}CO_2$). To account for the lag in the $\dot{V}O_2$ during the incremental test, two-thirds of the ramp rate (i.e., 2/3 × 30 W = 20 W) was deducted from the power output at gas exchange threshold and the peak power output attained in the test (19). The gas exchange threshold and $\dot{V}O_{2peak}$ were used to normalize the fixed resistance for the 3MTs. The resistance for the 3MT was applied using the linear factor function of the ergometer and was calculated as: linear factor = power output/preferred cadence$^2$ where the power was equal to the power output at the gas exchange threshold plus 50% of the difference between the gas exchange threshold and the peak power output.

On visit 2, participants completed a 3MT in a rested condition for familiarization purposes and, on visit 3, participants

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completed another 3MT in a rested condition as a control (C-3MT). The 3MT protocol began with a 3-min baseline of pedalling at 20 W. During a 5-s countdown during the baseline period, the participant was instructed to increase cadence to 110 to 120 rpm. Strong verbal encouragement was then given for an all-out effort from the onset of the 3MT but participants were not informed of the elapsed time. Instructions were given to reach peak power output as quickly as possible and to maintain the all-out effort throughout the test. The C-EP was calculated as the mean power output over the last 30 s of the C-3MT, and the C-WEP was calculated as the work done above EP during the C-3MT (10). The fixed constant power output imposed during the 2-h exercise bouts was calculated as the power output at the gas exchange threshold plus 25% of the difference between the gas exchange threshold and C-EP and was selected based on pilot testing which revealed that this power output was in the heavy-intensity domain and sustainable for 2 h.

On visits 4 and 5, participants completed a 3MT that was initiated immediately after 2 h of heavy-intensity, constant power output cycling (i.e., fatigued 3MT; F-3MT). The participants completed a 3-min baseline of cycling at 20 W, after which the power output was abruptly increased to the target power output for the 2 h of heavy-intensity exercise. Participants were instructed to maintain their preferred cadence throughout the 2 h. At the end of the 2-h bout, the power output was reduced to 20 W for 5 s, and the participant was instructed to accelerate cadence to 110 to 120 rpm, followed immediately by a 3MT as described above. During the 2-h constant power output test, the participants had a visible clock showing the time remaining. They were given the choice of listening to music during the 2-h test, with the same type of music replicated for all visits. The visible clock and the music were withdrawn 1 min before the F-3MT started. Participants were allowed to drink water *ad libitum* but not consume any food during the tests. Breath-by-breath gas exchange was recorded at the following time intervals: 10 to 15 min, 25 to 30 min, 55 to 60 min, 85 to 90 min, and 115 to 120 min and continuously through the F-3MT. F-EP and F-WEP were estimated from the F-3MT using the same procedures as for C-3MT. Before and after the test, participants were weighed in minimal clothing to assess changes in body mass.

**Experiment 2: Changes in 3MT parameters and physiological responses to exercise above and below EP after 2-h heavy-intensity exercise.** The 12 participants who volunteered for the second experiment attended the laboratory on 6 occasions over an approximately 4-wk period. Visits 1 to 4 were identical to those described above for Experiment 1. F-EP and F-WEP were estimated from the F-3MT completed during visit 4. During visits 5 and 6, a sub-set of nine participants completed in a randomized, counterbalanced order, a 2-h constant power output heavy-intensity exercise bout followed immediately by a constant power output exercise test at F-EP − 15 W (<F-EP) and F-EP +15 W (>F-EP). The range of ± 15 W was chosen based on the protocol of Burnley et al. (9). The <F-EP and >F-EP bouts were continued until the limit of tolerance ($T_{lim}$) or up to 30 min, whichever came first. Participants were not informed of the power outputs applied. $T_{lim}$ during the <F-EP and >F-EP bouts, when this was less than 30 min, was determined when cadence fell more than 10 rpm below the preferred cadence despite strong verbal encouragement. During the <F-EP and >F-EP constant power output exercise bouts, breath-by-breath gas exchange was recorded continuously while blood [lactate] was measured at rest, at 2, 4, and 8 min, and every 4 min thereafter until (and at) the end of exercise.

**Measurements (both experiments).** During all tests, pulmonary gas exchange was measured breath-by-breath and averaged over 10-s intervals. Participants wore a nose clip and breathed through a low dead space (90 mL), low resistance (0.75 mm $Hg \cdot L^{-1} \cdot s^{-1}$ at 15 $L \cdot s^{-1}$) mouthpiece and impeller turbine assembly (Jaeger Triple V). The inspired and expired gas volume and gas concentration signals were sampled continuously at 100 Hz (Jaeger Oxycon Pro, Hoechberg, Germany) via a capillary line connected to the mouthpiece. The analyzers were calibrated before each test with gases of known concentration and the turbine volume transducer was calibrated using a 3-L syringe (Hans Rudolph, MO). The volume and concentration signals were time-aligned by accounting for the delay in capillary gas transit and analyzer rise time relative to the volume signal.

The baseline $\dot{V}O_2$ during all visits was defined as the mean value over the final minute of the 3-min of unloaded pedalling. HR was recorded continuously during all visits at a frequency of 0.2 Hz (Garmin FR70; Garmin Ltd, Schaffhausen, Switzerland) and cadence and power output were recorded at 5 Hz through each visit. Fingertip blood samples (~25 μL) were collected into capillary tubes and analyzed promptly for blood [lactate] using an automated lactate analyzer (Stat2300; Yellow Spring Instrument, Yellow Springs, OH).

**Statistical analyses.** For experiment 1, the reliability of the F-EP and F-WEP were determined using intraclass correlation coefficients, the typical error of estimate, and the Bland–Altman criterion which states that at least 95% of the differences between test 1 and test 2 must lie between the mean difference in the variable in test 1 and the variable in test 2 ± 2 SD. Coefficients of variation (%) were calculated as typical error relative to the parameter estimate. For experiment 2, differences in C-EP and F-EP, C-WEP and F-WEP, total work done (TWD) and peak power output measured during the C-3MT and F-3MTs were assessed using paired sample *t*-tests. Relationships between changes in physiological variables during the 2-h heavy-intensity exercise test and changes in EP and WEP were assessed using Pearson product moment correlation coefficients. One-way ANOVA with repeated measures were used to assess differences in the $\dot{V}O_{2peak}$ during the ramp test, C-3MT and F-3MT, as well as differences in respiratory gas exchange variables and blood [lactate] during the <F-EP and >F-EP tests. Statistical significance was accepted at $P < 0.05$ level. Data are reported as mean ± SD unless otherwise stated.

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## RESULTS

**Experiment 1: Reliability of 3MT parameters after 2-h heavy-intensity exercise.** The $\dot{V}O_{2peak}$ in the ramp incremental test was 4.32 ± 0.43 L·min$^{-1}$, and the peak power output was 392 ± 36 W. F-EP estimated from the repeated F-3MTs were not different (test 1: 273 ± 52 W, test 2: 276 ± 58 W; 95% confidence interval 5, −11 W; $P = 0.35$). The intraclass correlation coefficient for F-EP between the two tests was $r = 0.99$ ($P < 0.001$), and the typical error was 5 W (2%) (Fig. 1A, B). F-WEP estimated from the two F-3MTs were not different (test 1, 12.4 ± 4.3 kJ; test 2, 12.8 ± 4.3 kJ; 95% confidence interval, 0.3 to −1.2; $P = 0.18$). The intraclass correlation coefficient for F-WEP between the two tests was $r = 0.99$ ($P < 0.001$), and the typical error was 0.8 kJ (6%) (Fig. 1C, D). The Bland–Altman criterion for test–retest reliability was satisfied for both F-EP (Fig. 1B) and F-WEP (Fig. 1D). Total work done was not different between test 1 (61.3 ± 7.5 kJ) and test 2 (62.4 ± 3.3 kJ; $P = 0.25$). The peak power output was not different between test 1 (826 ± 289 W) and test 2 (853 ± 318 W; $P = 0.47$).

**Experiment 2: Changes in 3MT parameters and physiological responses to exercise above and below EP after 2-h heavy-intensity exercise.** The $\dot{V}O_{2peak}$ in the ramp incremental test was 4.38 ± 0.53 L·min$^{-1}$, and the peak power output was 398 ± 45 W. During the 2-h constant power output test, the relative intensity increased from 10 to 15 min (64% ± 5% $\dot{V}O_{2peak}$) to 115 to 120 min (68% ± 5% $\dot{V}O_{2peak}$; $P < 0.05$; Fig. 2). Cadence decreased over the 2-h test from the first 5 min (84 ± 7 rpm) to the last 5 min (78 ± 8 rpm; $P < 0.001$).

The power output and $\dot{V}O_2$ profiles during the C-3MT and F-3MT are shown in Figure 3 ($n = 12$). There were no differences in $\dot{V}O_{2peak}$ between the C-3MT (4.22 ± 0.61 L·min$^{-1}$), the F-3MT (4.29 ± 0.53 L·min$^{-1}$), and the ramp incremental test ($P > 0.05$). F-EP was 8% lower (282 ± 52 W) compared with C-EP (306 ± 56 W; $P = 0.002$; Fig. 4A) and F-WEP (14.7 ± 4.9 kJ) was 20% lower compared with C-WEP (18.3 ± 4.1 kJ; $P = 0.02$; Fig. 4B). F-TWD (65.3 ± 10.5 kJ) was lower compared with C-TWD (73.3 ± 11.6 kJ; $P = 0.001$; Fig. 4C). There was no difference in peak power output between C-3MT (1040 ± 209 W) and F-3MT (1005 ± 318 W; $P = 0.63$; Fig. 4D). There were no significant correlations between changes in $\dot{V}O_2$ (in absolute or relative terms) during the 2-h heavy-intensity exercise test and changes in either EP or WEP.

The constant power outputs for the <F-EP and >F-EP tests ($n = 9$ for whom F-EP was 291 ± 50 W) were 276 ± 50 W and

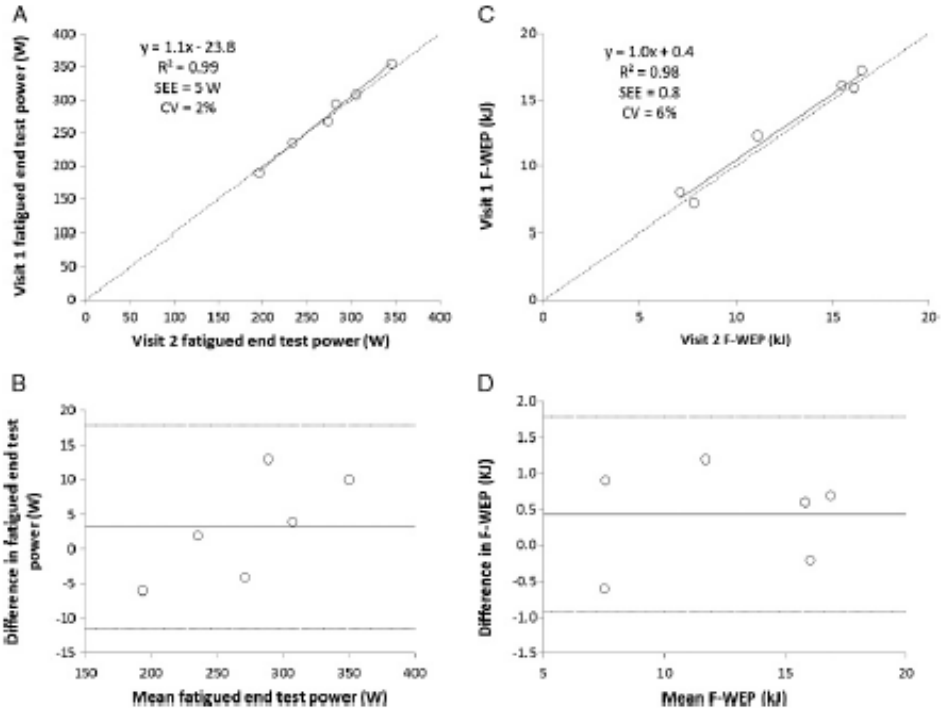


**FIGURE 1—Correlation and Bland–Altman analysis for the difference in end test power (panels A and B) and work done above end test power (F-WEP) (panels C and D) during the 3-min all-out test after 2 h of heavy-intensity exercise. In panels A and C the *solid line* is the best-fit linear regression and the dashed line the line of identity. In panels B and D, the solid horizontal line represents the mean difference between the two measurements and the *dashed line* represents the 95% limits of agreement between measurements.**

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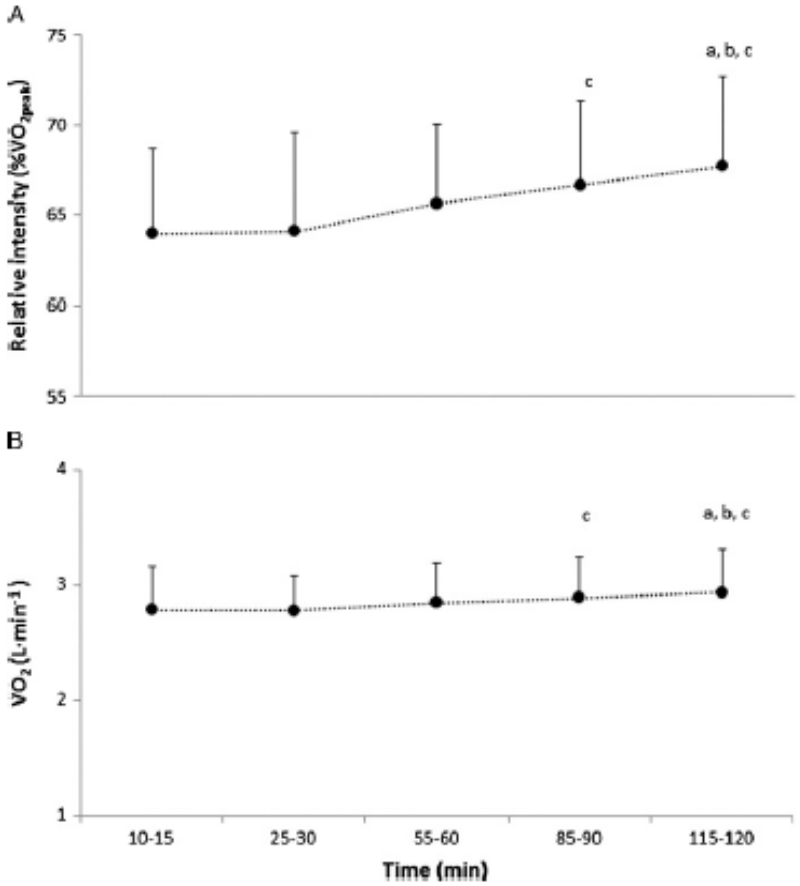


**FIGURE 2—Group mean relative intensity (% $\dot{V}O_{2peak}$) (A) and absolute pulmonary $\dot{V}O_2$ (B) during 2 h of heavy-intensity cycling. a, Different from 10 to 15 min ($P < 0.05$); b, different from 25 to 30 min ($P < 0.05$); c, different from 55 to 60 min ($P < 0.05$).**

306 ± 50 W, respectively. Only three of the nine participants completed the prescribed 30 min of exercise during the <F-EP constant power output test (group mean, 18.2 ± 10.7 min): of the six participants who did not complete 30 min of exercise, one participant reached exhaustion after 25 min, and five participants reached exhaustion between 7 and 18 min (Fig. 5). In the >F-EP test, participants reached exhaustion in 8.8 ± 6.2 min (range, 4–22 min; Fig. 5).

$\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, RER, HR, and blood [lactate] increased from the end of the 2-h exercise bout to the end of exercise in both the >F-EP and <F-EP tests ($P < 0.05$). The $\dot{V}O_2$ and blood [lactate] responses of the group and of a representative participant are shown in Figure 6. The $\dot{V}O_{2peak}$ was not different between <F-EP (3.93 ± 0.50 L·min$^{-1}$) and >F-EP (4.05 ± 0.41 L·min$^{-1}$; $P = 0.36$) but the $\dot{V}O_{2peak}$ values in both tests were lower than the $\dot{V}O_{2peak}$ measured in the ramp incremental test ($P < 0.05$). There was no difference in end-exercise HR between the <F-EP and >F-EP tests and the ramp incremental test ($P > 0.05$). There were no differences in $\dot{V}_E$ and blood [lactate] immediately before or at the end of exercise between the <F-EP and >F-EP tests ($P > 0.05$). $\dot{V}CO_2$ and RER were not different at the onset of the <EP and >EP tests ($P > 0.05$), but end-exercise $\dot{V}CO_2$ and RER were higher in >F-EP compared with <F-EP ($P < 0.05$). Body mass decreased during both the <F-EP test (Pre 76.1 ± 7.2, Post 75.1 ± 6.9 kg; $P < 0.05$) and the >F-EP test (Pre 76.0 ± 7.1, Post 75.2 ± 6.9 kg; $P < 0.05$), but there was no difference between tests ($P > 0.05$).

## DISCUSSION

This is the first study to investigate the influence of prolonged endurance exercise on the parameters of the power–time relationship (CP and $W'$) as estimated using the 3MT. Consistent with our experimental hypotheses: 1) the F-EP and F-WEP were reliable; and 2) both EP and WEP were significantly reduced, after 2 h of heavy-intensity exercise. However, in contrast to our hypothesis, 3) most participants were unable to exercise for longer than 20 min in the <F-EP test and $\dot{V}O_{2peak}$ achieved in the >F-EP test was significantly lower than $\dot{V}O_{2peak}$ measured in the ramp incremental test. Moreover,

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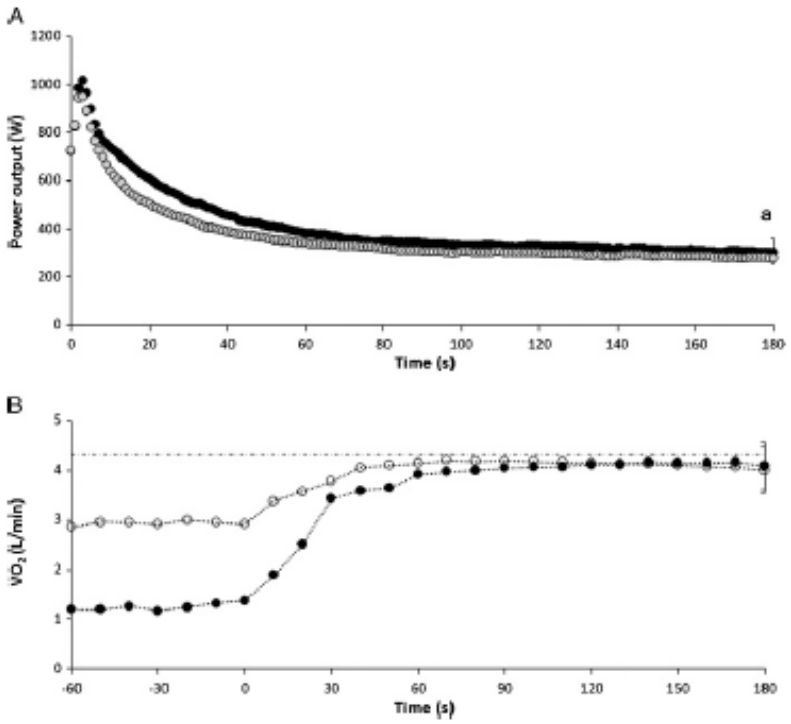


**FIGURE 3—Group mean power output (A) and pulmonary $\dot{V}O_2$ (B) during the 3-min all-out test measured with no prior exercise (black symbols) and after 2 h of heavy-intensity exercise (white symbols). To aid clarity, error bars (SD) are shown for end-exercise time points only. The *dashed line* in panel B indicates $\dot{V}O_{2peak}$ measured in the ramp incremental test. a, different from the control 3-min all-out test at 180 s ($P < 0.05$).**

while steady-state, submaximal values for $\dot{V}O_2$ and blood [lactate] were attained in the <F-EP test, consistent with the physiological responses that would be expected for exercise performed in the heavy-intensity domain, $\dot{V}O_2$ did not exhibit typical "slow component" behavior and blood [lactate] peaked at just ~4 mM in the >F-EP test, which is not consistent with the physiological responses emblematic of severe-intensity exercise. These novel observations indicate that, although EP (and, by extension, CP) falls appreciably after 2 h of heavy-intensity exercise, with important implications for understanding and predicting endurance performance, the characterization of CP, specifically the physiological responses to ostensibly severe-intensity exercise, may be different to that measured in a control (i.e., no prior exercise) condition.

**Reliability of 3MT parameters after 2-h heavy-intensity exercise.** The completion of 2-h heavy-intensity exercise before a 3MT did not compromise the reliable determination of EP and WEP. Indeed, EP and WEP estimates from the first and second tests did not differ (coefficient of variation, 2% and 6%, respectively) and were highly correlated ($r = 0.99$). This is similar to the reliability of the 3MT measured when participants have not undertaken prior exercise (9). Similarly, the $\dot{V}O_{2peak}$ values achieved during the ramp incremental test and the F-3MT were not significantly different, which is in accordance with previous research conducted when the 3MT is completed with no preceding exercise (9,10,20). High reliability of EP and WEP in the F-3MT provides confidence in the sensitivity of the test for assessing changes in EP and WEP after 2 h of heavy-intensity exercise.

**Changes in 3MT parameters after 2-h heavy-intensity exercise.** The EP, WEP, and TWD all declined in F-3MT compared with C-3MT. Specifically, there was an 8% reduction in F-EP compared with C-EP and a 20% reduction in F-WEP compared with C-WEP. This is an important novel finding which indicates that changes in CP and $W'$ over time need to be considered when performance after prolonged, fatiguing, endurance exercise is predicted using CP and $W'$ measured from tests completed in a rested state. An 8% reduction in CP over time will mean that a given constant power output will require a greater fraction of the highest sustainable oxidative metabolic rate (which is associated with the CP; 1, 4, 7), and might even result in a participant traversing CP, such that exercise which was initially heavy-intensity becomes severe-intensity, with detrimental effects on performance (17,18).

Several factors may contribute to the lower EP and WEP after completing 2 h of heavy-intensity exercise. Although the $O_2$ cost of sustaining the heavy-intensity constant power output increased over the 2-h exercise bout (from ~64% to ~68% of initial $\dot{V}O_{2peak}$), changes in $\dot{V}O_2$ with time during the 2-h exercise bout were not significantly correlated with changes in EP or WEP. The reduction in the EP, and WEP,

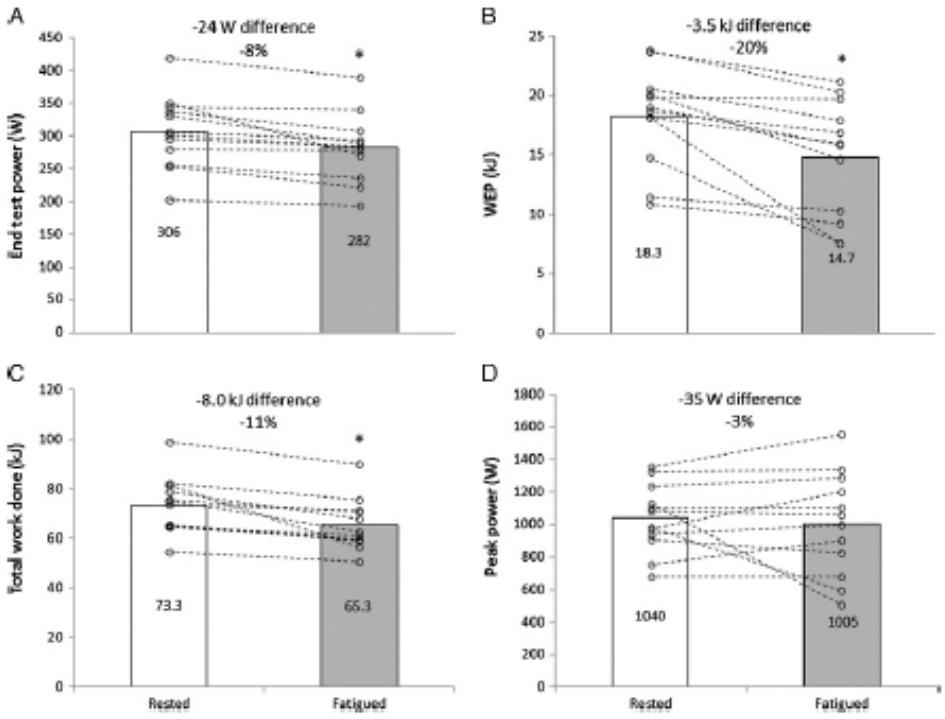


**FIGURE 4—Group mean end test power (A), work done above end test power (B), total work done (C) and peak power output (D) during the 3-min all-out test in a rested and fatigued state. *Dashed lines* represent individual responses. *Different from rested condition ($P < 0.01$).**

after completing 2 h of heavy-intensity exercise could be linked to greater central fatigue (14,21). Indeed, it has been previously reported that, during the single-leg equivalent of a 3MT, torque declines concomitantly with central fatigue development until critical torque is attained (15). Simultaneously, prolonged submaximal exercise, at a similar intensity and duration to that imposed in this study, has been reported to lower glycogen content in both type I and type II skeletal muscle (12,13). Because the proportion of type I muscle fibers in the *m. vastus lateralis* is positively correlated with CP (8), the decline in glycogen in this fiber population might have contributed to the lower F-EP compared to C-EP. Although the proportion of type II fibers in the *m. vastus lateralis* is not significantly correlated with $W'$ (8), and the determinants of $W'$ are considered to be complex and multifactorial (1,8,22,23), reduced glycogen levels in both type I and type II fibers might be anticipated to impact on WEP given the specific motor unit recruitment profile exhibited during the 3MT (20,23).

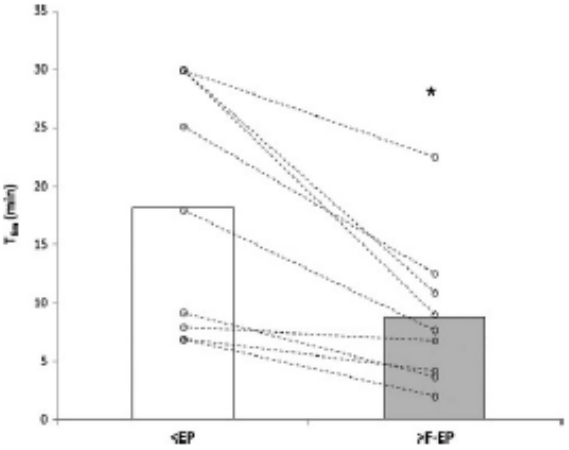


**FIGURE 5—Time to the limit of tolerance during exercise performed below (<F-EP) and above (>F-EP) the end test power measured in a 3-min all-out test, after 2 h of heavy-intensity exercise. *Dashed lines* represent individual responses. *Different from <F-EP test ($P < 0.01$).**

Miura et al. (16) reported that glycogen depletion, achieved via dietary restriction and an exercise protocol performed the evening before conventional CP prediction trials, resulted in a significant reduction in $W'$ but no significant change in CP. The explanation for the apparent discrepancy between that study and the present one is unclear but it is possible that the extent of glycogen depletion was greater in our study or that CP is more sensitive to the influence of exercise-induced glycogen depletion when such exercise occurs in closer proximity to its measurement (i.e., immediately preceding compared to the previous evening). Interestingly, although WEP was significantly reduced by 2 h of heavy-intensity exercise in the present study, the peak power output attained was not different between the C-3MT and the F-3MT. This is consistent with the suggestion that $W'$ is related, in part, to muscle

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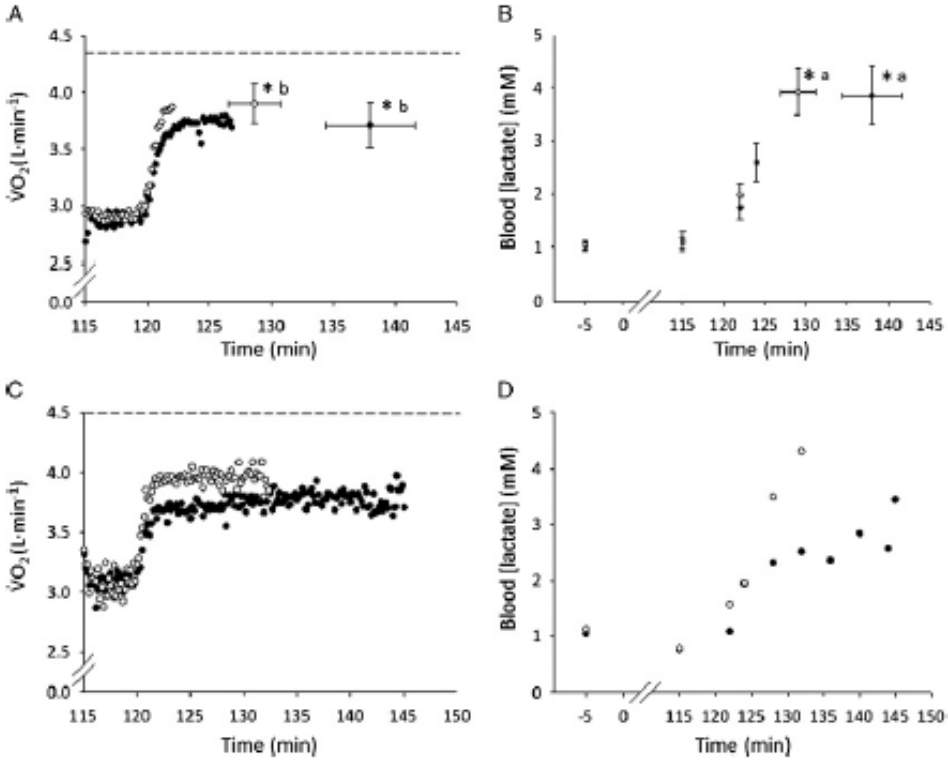


**FIGURE 6—Group mean pulmonary $\dot{V}O_2$ (panel A) and blood [lactate] (panel B), and representative participant $\dot{V}O_2$ (panel C) and blood [lactate] (panel D) responses for exercise performed below (<F-EP; *black symbols*) and above (>F-EP; *white symbols*) the end test power measured in a 3-min all-out test, after 2 h of heavy-intensity exercise. Error bars (SE) are shown for end-exercise time points in panels A and B. The *dashed lines* in panels A and C indicates $\dot{V}O_{2peak}$ measured in the ramp incremental test. *Different from measurements made at the end of 2-h heavy-intensity exercise ($P < 0.05$); a, different from baseline measurements ($P < 0.05$); b, different from measurements made in the ramp incremental test ($P < 0.05$).**

glycogen content (16), but suggests that peak power output is related more to other factors, such as muscle high-energy phosphate content (11,24), which would not be expected to be substantially altered by 2 h of submaximal exercise (5).

It has recently been reported that "mental fatigue," induced by the completion of demanding cognitive function tasks, can reduce $W'$, but not CP, when these parameters are assessed using conventional prediction trials (25). In the present study, both EP and WEP, as established with the 3MT, were reduced after 2 h of heavy-intensity exercise. Whether mental fatigue can develop during prolonged endurance exercise remains to be investigated but, if present in the current study, it apparently did not prevent participants from exerting maximal effort in the F-3MT because they achieved similar peak power output and $\dot{V}O_{2peak}$ values to those measured in the C-3MT.

**Changes in physiological responses to exercise above and below EP after 2 h heavy-intensity exercise.** In addition to examining changes in EP and WEP after 2 h of heavy-intensity exercise, we investigated the physiological significance of these changes and their implications for exercise tolerance. In particular, we aimed to establish whether F-EP continued to represent CP, based on its established characteristics during tests completed without prior fatiguing exercise (4,8), by investigating the physiological responses to exercise <F-EP and >F-EP. It has been consistently reported that <CP exercise can be sustained for 30 min at a submaximal $\dot{V}O_2$ and with elevated but stable blood [lactate], whereas >CP exercise is associated with progressive increases in $\dot{V}O_2$ and blood [lactate] over time and limited exercise tolerance (4,8).

As expected, $T_{lim}$ was significantly shorter for >F-EP compared to <F-EP. However, the $\dot{V}O_{2peak}$ achieved in the >F-EP test was significantly lower than the $\dot{V}O_{2peak}$ measured in the ramp incremental test, and more than half of the participants failed to sustain exercise for longer than 20 min during the <F-EP test. Apart from the failure of most subjects to complete the prescribed exercise duration of 30 min, the physiological responses to the <F-EP test were consistent with what might be expected for exercise performed in the heavy-intensity domain (4,8), with $\dot{V}O_2$ and blood [lactate] attaining steady state, submaximal values (Fig. 6). A striking feature of our results, however, was that the physiological responses to the >F-EP test were not consistent with what has been reported previously for exercise performed in the severe-intensity domain (4,8). Specifically, during the >F-EP test, there was limited evidence of non–steady state "slow component" behaviour, with the rise in $\dot{V}O_2$ being truncated at a lower than expected $\dot{V}O_{2peak}$, and the rise in blood [lactate] being blunted

and reaching only 4 mM at $T_{lim}$ (Fig. 6). These responses are more akin to those that might be expected during the so-called extreme-intensity exercise (26,27), albeit that maximal exercise duration in this domain is considerably shorter (<2–3 min) when undertaken in the fresh state (26,27). One interpretation of these results is that, after prolonged heavy-intensity exercise, the EP no longer demarcates the boundary between the heavy- and severe-intensity exercise domains or that the range of the severe-intensity domain is markedly diminished. Further research is required to assess whether, and how, prolonged exercise might alter conventional assessments of CP, $W'$, and $\dot{V}O_{2peak}$, as well as the potential ergolytic effects of such changes.

Participants were permitted to consume water during the 2 h of heavy-intensity exercise, and the decrement in body mass (~0.9 kg) was not different between the tests. Although the maximum HR achieved in the incremental, <F-EP and >F-EP tests was not different, and although the influence of cardiovascular drift on changes in $\dot{V}O_{2peak}$ was likely attenuated by fluid ingestion during the 2 h of heavy-intensity exercise (28), it is possible that the reduced $\dot{V}O_{2peak}$ in >F-EP was, at least in part, related to a reduced stroke volume. However, this cannot explain why the $\dot{V}O_{2peak}$ measured in the initial ramp incremental test could be achieved in the F-3MT but not the >F-EP test. It is possible that exercise duration (3 min vs ~9 min), and/or pacing strategy (all-out vs constant power output) and associated motor unit recruitment patterns (23), influence whether or not the same $\dot{V}O_{2peak}$ can be achieved when exercise is initiated after 2-h heavy-intensity exercise compared with a rested condition.

As discussed above in relation to changes in EP and WEP, prolonged heavy-intensity exercise may have resulted in peripheral metabolic or neuromuscular changes which compromised the ability of participants to sustain higher-intensity exercise and to achieve the same $\dot{V}O_{2peak}$ during the >F-EP test. It is possible that the lower $\dot{V}O_{2peak}$ in the >F-EP test, and the failure of most participants to complete more than 20 min of exercise in the <F-EP test, is related to the effects of a combination of lower muscle glycogen content and increased central fatigue on skeletal muscle activation. It is well documented that declining muscle [glycogen] is linked to fatigue during prolonged submaximal exercise at similar intensities to those applied in the current study (29,30). Combined with other metabolic perturbations in skeletal muscle after prolonged heavy-intensity exercise (5,31), the lower [glycogen] in type I and II skeletal muscle (12,13) would be expected to impede the recruitment of additional skeletal muscle fibers to contribute to force production when the power output was increased at the onset of the <F-EP and >F-EP tests. Failure to recruit additional muscle fibers, and/or a reduced ability of the recruited fibers to generate power due to peripheral fatigue development (5–8), might have prevented the completion of >20 min exercise in the <F-EP test and the attainment of $\dot{V}O_{2peak}$ in the >F-EP test. The attenuation or absence of the $\dot{V}O_2$ slow component, as well as the low peak blood [lactate] attained at $T_{lim}$, in the >F-EP test is consistent with possible effects of prolonged heavy-intensity exercise on type II fiber recruitment or function (1) and/or a blunted ability to resynthesize ATP via substrate-level phosphorylation (7,8,22,23). Moreover, since $W'$ (22) and WEP (23) have been related to the amplitude of the $\dot{V}O_2$ slow component, the truncation of the $\dot{V}O_2$ response at a lower-than-expected $\dot{V}O_{2peak}$ in the >F-EP test is consistent with the 20% reduction in WEP measured in the current study.

It should also be considered that 2 h of heavy exercise may have adversely influenced participants' motivation to exercise to exhaustion in the longer-duration, open-ended constant-power output tests (<F-EP and >F-EP), compared with the 3MT. The relatively low blood [lactate] measured at $T_{lim}$ in the >F-EP test (~4 mM) is consistent with Salam et al. (25) who reported that preexercise completion of mentally fatiguing tasks reduced $T_{lim}$ and peak blood [lactate] during CP prediction trials. However, the low blood [lactate] at $T_{lim}$ during the >F-EP test might also be explained by muscle glycogen depletion caused by the 2-h heavy-intensity exercise bout. We acknowledge that a potential limitation of our study was that <EP and >EP tests were not undertaken under control conditions in the present study; however, the physiological responses to such tests have been well described previously (4,5,8,10). Further research involving, for example, assessment of muscle activation, muscle [glycogen], and rating of perceived exertion, is necessary to provide greater insight into the mechanisms responsible for the reductions in EP, WEP and exercise tolerance below and above EP after prolonged exercise.

**Practical implications.** The 8% reduction in EP, along with the observation that $T_{lim}$ was lower in the >F-EP test than the <F-EP test, suggests that the decline in CP after prolonged heavy-intensity exercise will limit endurance exercise performance by lowering the sustainable race pace. It was recently reported that élite athletes sustained ~96% of CS (as estimated from race performances at shorter distances) during a marathon race (18), that is, at the upper end of the heavy-intensity domain. Assuming that CS during running declines to a similar extent to CP during cycling, the results of the present study indicate that the value of 96% may be an underestimation. If an athlete maintains a constant speed during a long distance race, he or she will gradually get closer to and will eventually encroach upon CS, and presumably experience an increasing physiological strain and sense of effort with time; alternatively, if an athlete maintains a constant fraction of CS, then his or her speed will naturally fall with time as CS declines. It should also be pointed out that the decline in $W'$ after heavy-intensity exercise would limit the ability of an athlete to initiate an acceleration or respond to competitors attempting to "break away from the pack," most notably during a sprint finish. The present study also has implications for the prediction of endurance performance from exercise tests completed in a rested state: our results indicate that performance during long-duration endurance events might depend not only on the value of key physiological variables at baseline (32,33) but also on the extent to which these variables deteriorate as

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exercise proceeds. In this light, resistance to the loss of CS (which, in turn, may be related to a better maintenance of mechanical and metabolic efficiency) may be an important characteristic of the athlete who is eventually capable of completing a marathon in less than 2 h (34). Further research is required to resolve the mechanisms responsible for changes in the power–duration relationship after prolonged heavy-intensity exercise and to determine whether these effects can be mitigated by training, pacing, mechanical, or nutritional interventions.

## CONCLUSIONS

This study shows that EP and WEP can be reliably determined from a 3MT completed after 2 h of heavy-intensity exercise. Compared with C-3MT, EP and WEP were lowered in F-3MT which is suggestive of a lowering of CP and $W'$, respectively. However, in the >F-EP test, $\dot{V}O_{2peak}$ was lower than that observed in the initial ramp incremental test, and in the <F-EP test, the majority of participants were unable to complete more than 20 min of exercise. Therefore, while EP declines after 2 h of heavy-intensity exercise and is associated with impaired exercise performance, the physiological and functional definition of EP appears to be fundamentally different to that which has been well described during tests completed without prior exercise. Further research is warranted to resolve the physiological mechanisms for, and the performance implications of, changes in the parameters of the power–time relationship after prolonged heavy-intensity exercise.

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APPLIED SCIENCES

## Summary of chapter 5

Chapter 5 showed that the 3MT is a reliable test to estimate EP and WEP after 2 h of heavy-intensity exercise. However, it was not able to determine whether EP and WEP were estimated after 2 h of heavy-intensity exercise using the 3MT would correlate with the CP and W′ estimated using the conventional model. Therefore the next study was conducted to address this. Additionally, chapter 6 sought to investigate the relationship between muscle glycogen depletion and changes in EP and WEP after 2 h of heavy-intensity exercise.

# Chapter 6. Changes in the power-duration relationship following prolonged exercise: estimation using conventional and all-out test protocols and the relationship to muscle glycogen

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## ABSTRACT

It is not clear how the parameters of the power-duration relationship (critical power (CP) and W′) are influenced by the performance of prolonged endurance exercise. We used severe-intensity prediction trials (conventional protocol) and the 3-min all-out test (3MT) to measure CP and W′ following 2 h of heavy-intensity cycling exercise and took muscle biopsies to investigate possible relationships with changes in muscle glycogen concentration ([glycogen]). Fourteen participants completed a rested 3MT to establish end-test power (Control-EP) and work done above EP (Control-WEP). Subsequently, on separate days, immediately following 2 h of heavy-intensity exercise, participants completed a 3MT to establish Fatigued-EP and Fatigued-WEP and three severe-intensity prediction trials to the limit of tolerance ($T_{lim}$) to establish Fatigued-CP and Fatigued-W'. A muscle biopsy was collected immediately before and after one of the 2-h exercise bouts. Fatigued-CP (256 ± 41 W) and Fatigued-EP (256 ± 52 W), and Fatigued-W' (15.3 ± 5.0 kJ) and Fatigued-WEP (14.6 ± 5.3 kJ), were not different ($P$>0.05), but were ~11% and ~20% lower than Control-EP (287 ± 46 W) and Control-WEP (18.7 ± 4.7 kJ), respectively ($P$<0.05). The change in muscle [glycogen] was not significantly correlated with the changes in either EP ($r$ = 0.19) or WEP ($r$ = 0.07). The power-duration relationship is substantially impacted by prolonged endurance exercise. The 3MT provides valid estimates of CP and W′ following 2 h of heavy-intensity exercise but the changes in these parameters are not primarily determined by changes in muscle [glycogen].

**Key words:** CRITICAL POWER, FATIGUE, PERFORMANCE, METABOLISM

## 6.1 Introduction

The power-asymptote of the hyperbolic power-duration relationship, critical power (CP), separates the 'severe' from the 'heavy' exercise intensity domains (22, 25, 32). During exercise performed within the heavy-intensity domain (<CP), a steady-state in oxygen uptake ($\dot{V}O_2$) can be obtained and this is accompanied by stable muscle [phosphocreatine] ([PCr]), pH, [lactate] and [inorganic phosphate] ([$P_i$]) responses (4, 23, 32, 36). In contrast, during exercise performed within the severe-intensity domain (>CP), the development of a $\dot{V}O_2$ 'slow component' results in the attainment of maximal oxygen uptake ($\dot{V}O_{2max}$), muscle [PCr], pH, [lactate] and [$P_i$] exhibit non-steady state profiles (4, 23, 36), and exercise tolerance is correspondingly limited (32). The amount of work that can be performed >CP before the limit of tolerance ($T_{lim}$) is represented by the curvature constant (W′) of the power-duration relationship with $T_{lim}$ being reached when W′ is fully expended (i.e. W′ = 0 kJ; 11, 25). Knowledge of CP and W′ permits accurate prediction of performance for various distances and durations of exercise (21, 22, 40).

CP and W′ are conventionally estimated by measuring $T_{lim}$ during a series (~3-4) of constant-power (P), severe-intensity prediction trials performed on separate days, and modelling the power-duration relationship (18). Alternatively, CP and W' can be estimated from a single 3-min all-out cycle ergometer test against fixed resistance (3MT) where, provided that $\dot{V}O_{2max}$ is attained and maintained, the mean power output over the last 30 s of the test (end-test power; EP) reflects the CP and the work done above EP (WEP) reflects the W' (28, 37, 38). We have previously shown that EP and WEP derived from the 3MT decreased by 8% and 20%, respectively, after 2 h of heavy-

intensity exercise (8). These effects would be expected to have significant implications for performance during events lasting ≥2 h (21), and also for the prediction of such performance from exercise tests conducted in a fresh state, i.e. without preceding fatiguing exercise (8). However, while the EP and WEP provide valid and reliable estimates of the CP and W′ when exercise tests are commenced from a rested baseline (5, 28, 37, 38; cf. 26), it is not known whether this close agreement between the parameter estimates derived from the two different protocols is maintained following the performance of long-duration endurance exercise. It is possible, for example, that the parameters of the power-duration relationship as derived from the conventional protocol (continuous constant-power prediction trials to $T_{lim}$ of ~2-15 min duration) and the 3MT protocol (all-out exercise for 3 min) are affected differentially by factors related to the development of fatigue during long-duration endurance exercise.

It is well established that fatigue during prolonged exercise at intensities equivalent to 70-75% of $\dot{V}O_{2max}$ is associated with the attainment of low muscle [glycogen] (9, 16). It is therefore possible that the reductions of EP and WEP measured in a fatigued compared to a rested state (8) are related to changes in muscle [glycogen]. Consistent with this, it has been reported that W' is reduced by ~20% when glycogen stores are depleted by dietary carbohydrate restriction (24). Given that CP reflects the highest sustainable oxidative metabolic rate (23, 32, 36), it is possible that the impaired endurance performance associated with glycogen depletion is reflected in a reduced CP, but this has not been formally investigated. Resolving whether a change in the power-duration relationship following 2 h of heavy-intensity exercise is related to muscle glycogen depletion would not only provide novel mechanistic insight into this

phenomenon but might also inform strategies to modulate the performance impact of long-duration endurance exercise.

The purpose of this study was to determine CP and W′ derived from the conventional protocol following 2 h of heavy-intensity exercise, assess the level of agreement with EP and WEP derived from the 3MT, and evaluate the relationships between muscle glycogen depletion and changes in EP and WEP. We hypothesized that, following 2 h of heavy-intensity exercise: 1) 'Fatigued' CP and W′ (Fatigued-CP and Fatigued-W') estimated using the conventional protocol would be significantly lower compared to the values estimated without the performance of prior exercise; 2) Fatigued-CP and Fatigued-W' estimated using the conventional protocol would not be different from the Fatigued-EP and Fatigued-WEP estimated using the 3MT; and, 3) the reductions in EP and WEP would be correlated with the reduction in muscle [glycogen].

## 6.2 Methods

Fourteen male participants (mean ± SD: age, 31 ± 10 years; height, 1.79 ± 0.06 m; body mass, 79.2 ± 6.5 kg; $\dot{V}O_{2peak}$, 54.7 ± 5.4 ml·kg$^{-1}$·min$^{-1}$) volunteered to take part in the study. The study procedures were approved by the Institutional Research Ethics Committee and participants provided written informed consent prior to participation. All exercise tests were separated by a minimum of 24 h and the >2-h exercise bouts were separated by at least 72 h. Participants were instructed to avoid alcoholic drinks and strenuous exercise 24 h prior to testing. Participants completed a diet and exercise diary 48 h prior to their first visit. These diaries were photocopied and participants were

instructed to repeat the reported dietary and exercise behavior prior to each subsequent visit.

*Experimental procedures*

All exercise tests were conducted using the same electrically-braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). During all tests, except the 3MT, the participants cycled at a self-selected cadence. Cadence was not controlled during the 2-h exercise tests but participants were asked to maintain it to within ± 5 rpm during subsequent constant-power output tests. The ergometer seat and handlebars were adjusted for comfort during the first visit and settings were recorded and replicated for all subsequent visits. Participants attended the laboratory on eight occasions. During the first visit, participants performed a 30 W/min ramp incremental exercise test for the determination of $\dot{V}O_{2peak}$ and gas exchange threshold (GET). Initially, participants performed 3 min of 'unloaded' baseline cycling, after which the power output was increased by 30 W/min until $T_{lim}$, which was recorded once the cadence fell by >5 rpm below the participant's self-selected cadence. $\dot{V}O_{2peak}$ was determined as the highest 30-s rolling mean measured during the ramp incremental test. The GET was estimated using the methods described by Beaver et al. (1). $\dot{V}O_{2peak}$ and GET were used to calculate the resistance for the 3MTs and to normalize the power output during the 2-h heavy-intensity exercise bouts. The fixed resistance for the 3MTs was calculated using the equation: linear factor = power/(preferred cadence)$^2$ where power output was 50%$\Delta$ (i.e., GET plus 50% of the difference between the power outputs at GET and $\dot{V}O_{2peak}$) and preferred cadence was the cadence selected (rpm) during the ramp incremental test. The linear factor was 0.040 ± 0.005 (range 0.035 - 0.050) W/rpm$^2$. The linear factor

ensures that a particular cadence will produce a known power output. In the calculation of power outputs to be used during exercise tests, account was taken of the lag in $\dot{V}O_2$ relative to power output during ramp exercise (42).

On visits 2 (familiarization) and 3 (control visit), a single 3MT was completed. Participants started by performing a 3-min 'unloaded' baseline period. Then, 5 s before the all-out sprint commenced, the participants were asked to increase cadence to 110-120 rpm. For the entirety of the 3MT, participants were asked to cycle as quickly as possible. Strong verbal encouragement was given throughout the test but no information was provided on time elapsed. Control-EP was estimated as the mean power output over the last 30 s of the test and Control-WEP was defined as the work done above EP (22, 37). During the 2 h heavy-intensity exercise bout, participants cycled at 25%$\Delta$1 (i.e., where $\Delta$1 refers to the work rate at GET plus 25% of the difference between the work rate at GET and Control-EP). Pilot testing indicated that this power output (25%$\Delta$1) was challenging but sustainable for 2 h.

During visit 4, participants completed 2 h of heavy-intensity exercise followed by a 3MT (Fatigued-3MT). Prior to the start of the exercise test, participants provided a resting muscle biopsy sample (described below). The exercise protocol started with cycling at 20 W for 3 min, after which the power output abruptly increased to 25%$\Delta$1. Participants were instructed to maintain their preferred pedal cadence for the whole 2 h. They were allowed to consume water *ad libitum*. A clock indicating time remaining was visible during the 2 h exercise bout and participants were allowed to listen to music, but both the clock and the music were withdrawn 1 min prior to the start of the Fatigued-3MT. An end-exercise muscle biopsy was taken at 120 min and the Fatigued-3MT commenced

at 121 min. The Fatigued-3MT was administered as described for the Control-3MT, except that the baseline period of unloaded cycling was omitted. Pulmonary gas exchange data were recorded at the following time points: -3-15 min, 25-30 min, 55-60 min, 85-90 min, 115-120 min and continuously throughout the Fatigued-3MT. A blood sample was taken every 30 min during the 2-h heavy-intensity exercise bout for the analysis of blood [lactate], blood [glucose] and plasma potassium ($[K^+]$). Heart rate (HR) and cadence were obtained continuously over the entire exercise testing period. Fatigued-EP and Fatigued-WEP was estimated from the Fatigued-3MT using the same procedures as for the Control-3MT.

During visits 5-7, participants performed the same 2-h heavy-intensity exercise bout as in visit 4 but this was followed immediately by a severe-intensity, constant-power output prediction trial which was continued until $T_{lim}$. The purpose of completing these prediction trials was to determine the power-duration parameters in a fatigued state (Fatigued-CP and Fatigued-W') using the conventional protocol (e.g. 3). The power outputs for the three severe-intensity exercise bouts were calculated from the Fatigued-3MT (visit 4) to provide $T_{lim}$ values ranging between approximately 2 min and 15 min (a short, intermediate and long trial). During the prediction trials, participants were not informed of the power output applied or the time elapsed but were instructed to cycle for as long as possible. $T_{lim}$ was recorded when participants could not maintain their preferred cadence for >5 s. Breath-by-breath pulmonary gas exchange data were obtained from 5 min before the end of the 2 h heavy-intensity exercise bout until $T_{lim}$ during the severe-intensity prediction trials. A capillary blood sample for the determination of blood [lactate] was taken from the fingertip at the following time points

during the trials: -5 min, 2 min, 4 min, 8 min and every 4 min thereafter until $T_{lim}$, and at $T_{lim}$. Linear regression using the work-time ($W = CPt + W'$) and 1/time ($P = W' (1/t) + CP$) models, as well as the hyperbolic model ($T_{lim} = W' / (P – CP)$), were used to obtain 3 sets of Fatigued-CP and Fatigued-W' parameters from the prediction trials. The best individual fit of the 3 models was used for further analyses (3, 4).

On visit 8, participants completed a final 2 h heavy-intensity exercise bout, identical to visit 4-7, but followed immediately by a constant-power output test at 15 W below Fatigued-CP (<Fatigued-CP). This bout was completed to test the assumption that exercise <Fatigued-CP would result in physiological responses consistent with exercise in the heavy-intensity domain (5, 23, 32). The exercise bout lasted until $T_{lim}$ or for 30 min, whichever occurred sooner. Breath-by-breath pulmonary gas exchange data were recorded continuously from 115 min of the 2 h heavy-intensity exercise bout until the cessation of the protocol. Blood [lactate] was measured at the same time points as in visits 5-7.

*Pulmonary gas exchange and heart rate*

Pulmonary gas exchange was measured breath-by-breath and bin-averaged over 10-s periods. Participants wore an oro-nasal mask (Hans Rudolf 7450 Series V2$^{TM}$ Mask, CareFusion, Germany). The inspired and expired gas volume and gas concentration signals were sampled continuously at 100 Hz (Vyntus, CareFusion, Germany) via a capillary line connected to the mask. The analyzer was calibrated before each test with gases of known concentration and the turbine volume transducer was calibrated using a 3-L syringe (Hans Rudolph, MO). The volume and concentration signals were time-aligned by accounting for the delay in capillary gas transit and analyzer rise time relative

to the volume signal. The baseline $\dot{V}O_2$ during all tests was defined as the mean value over the final minute of the 3-min period of unloaded pedalling. Fat and carbohydrate oxidation rates were calculated from $\dot{V}O_2$ and carbon dioxide output ($\dot{V}CO_2$) using stoichiometric equations with the assumption that protein oxidation during exercise did not change (19).

Carbohydrate oxidation ($g \cdot min^{-1}$) = [4.21 ($\dot{V}CO_2$) – 2.692 ($\dot{V}O_2$)]

Fat oxidation ($g \cdot min^{-1}$) = [1.695 ($\dot{V}O_2$) – 1.701 ($\dot{V}CO_2$)]

HR was recorded every 5 s during all visits (Garmin FR70, Garmin Ltd, Schaffhausen, Switzerland).

*Muscle biopsies*

Muscle samples were obtained from an incision from the medial region of the *m. vastus lateralis* under local anesthesia (1% lidocaine) using the percutaneous Bergström needle biopsy technique under suction (2) . Muscle samples were taken at rest and immediately following 2 h of heavy-intensity exercise during visit 4. The post-exercise muscle biopsies were taken while participants remained on the cycle ergometer and snap frozen in liquid $N_2$ within ≤10 s of the completion of the exercise bout. Biopsy samples were stored at -80 °C for subsequent analysis.

*Muscle glycogen concentration*

Muscle samples were freeze-dried prior to dissection from connective tissue, fat and blood. Approximately 2 mg of dry weight muscle tissue was hydrolyzed in 500 µl of 1 M hydrochloric acid at 100°C for 3 h to release glycosyl units and immediately measured

using an automated glucose analyser to determine muscle [glycogen] (YSI 2900 Biochemistry Analyzer; Yellow Springs Instruments, Yellow Springs, OH), (33). The precision of this method of analysis within this physiological range (0.05 to 0.55 mmol/l) was checked by measuring the glucose concentration across a range of solutions made up using glucose diluted in hydrochloric acid; the measured vs. expected values lay on the line of identity with an $R^2$ of 0.99.

*Blood analyses*

During visit 4, blood samples were obtained from a cannula (Insyte-W; Becton Dickinson, Madrid, Spain) inserted in an antecubital vein. Samples were drawn at rest and at specific times during the 2-h heavy-intensity exercise bout. Blood samples were collected into a lithium-heparin vacutainer (Becton-Dickinson, New Jersey, USA). 200 µL of blood was immediately extracted and haemolyzed in 200 µL of Triton X-100 Solution (Triton X-100, Amresco, Salon, OH) and blood [glucose] and [lactate] were measured (YSI 2900 Biochemistry Analyzer; Yellow Springs Instruments, Yellow Springs, OH). The remaining blood was centrifuged at 4000 rpm for 10 min at 4°C. The plasma was extracted and frozen at -80°C and subsequently analysed for [$K^+$] using Stat Profile pHOx Ultra (Nova Biomedical, Waltham, MA, USA). All fingertip blood samples (~25 µl) (visit 5-8) were collected into capillary tubes and analysed within 30 s for blood [lactate] using an automated lactate analyser (YSI 2900 Biochemistry Analyzer; Yellow Springs Instruments, Yellow Springs, OH).

*Statistical analysis*

Errors associated with mathematical modelling of the CP and W' parameters from prediction trial data were quantified as standard error and expressed relative to the parameter estimate (coefficient of variation, CV%) for each individual. One-way ANOVA with repeated measurements were used to assess differences in Control-EP, Fatigued-EP and Fatigued-CP; Control-WEP, Fatigued-WEP and Fatigued-W'; and $\dot{V}O_{2peak}$ during the ramp test, Control-3MT and Fatigued-3MT. A one-way ANOVA with repeated measurements was also used to assess differences in $\dot{V}O_{2peak}$ alongside $HR_{max}$ between the ramp test, <Fatigued-CP, short, intermediate and long duration severe-intensity prediction trials, as well as differences in respiratory gas exchange variables, blood [lactate] and blood [glucose] during all visits. Differences in total work done as well as peak power output measured during the Control-3MT and Fatigued-3MTs were assessed using paired samples t-tests. Agreement between the power-duration parameters derived from different protocols was assessed using intra-class correlation coefficients and the Bland-Altman analysis. The difference in muscle [glycogen] between rest and following 2 h of heavy-intensity exercise was assessed using a paired samples t-test. Relationships between absolute muscle [glycogen], and changes in muscle [glycogen], over the 2-h heavy-intensity exercise test and the changes in EP and WEP were assessed using Pearson product moment correlation coefficients. Statistical significance was accepted when $P<0.05$. Data are reported as mean ± SD.

## 6.3 Results

The $\dot{V}O_{2peak}$ in the ramp incremental test was 4.31 ± 0.35 L·min$^{-1}$, and the peak power output was 368 ± 48 W. During the 2-h heavy-intensity exercise bout, the relative intensity increased from 10-15 min (65 ± 6 % $\dot{V}O_{2peak}$) to 115-120 min (72 ± 4 % $\dot{V}O_{2peak}$; $P$<0.05; Fig. 1) and the respiratory exchange ratio decreased from 10-15 min (0.86 ± 0.05) to 115-120 min (0.79 ± 0.05; $P$<0.05). There was a decrease in carbohydrate oxidation (10-15 min: 1.77 ± 0.73 g/min, 115-120 min: 1.17 ± 0.76 g/min) and an increase in fat oxidation (10-15 min: 0.68 ± 0.29 g/min, 115-120 min: 1.08 ± 0.30 g/min) during the 2-h exercise bout ($P$<0.05). HR increased over time during the 2-h exercise bout ($P$<0.05). Blood [lactate] and blood [glucose] did not change during the 2-h exercise bout; plasma [$K^+$] was elevated above the resting value at all time points ($P$<0.05) during the 2-h exercise bout but remained stable beyond 30 min (Table 1). HR throughout the 2-h exercise bouts and end-exercise blood [lactate] were not different between visits 4-8. Body mass fell by ~0.9 kg during the prolonged exercise tests with no difference between visits.

***Table 1.*** *Blood lactate, glucose and plasma potassium responses during 2 h of heavy-intensity exercise.*

| | 0 min | 30 min | 60 min | 90 min | 120 min |
|---|---|---|---|---|---|
| [Lactate] (mM) | 1.2 ± 0.6 | 1.6 ± 0.4 | 1.3 ± 0.3 | 1.4 ± 0.4 | 1.4 ± 0.4 |
| [Glucose] (mM) | 4.4 ± 0.9 | 4.2 ± 0.8 | 4.1 ± 0.6 | 4.1 ± 0.8 | 3.8 ± 0.6 |
| [$K^+$] (mM) | 4.0 ± 0.4 | 5.1 ± 0.3[a] | 5.3 ± 0.7[a] | 5.3 ± 0.5[a] | 5.0 ± 0.8[a] |

Values are means ± SD. a = significantly different from 0 min ($P$ < 0.05).

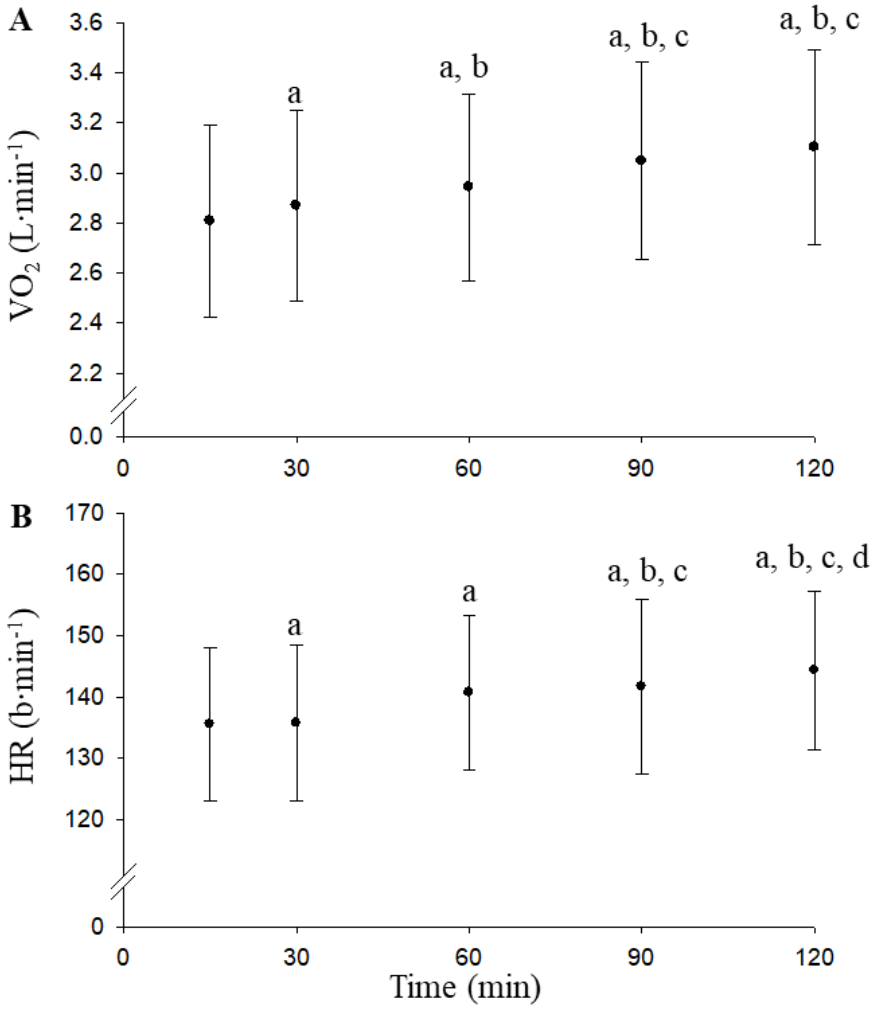


***Figure 1.*** *Group mean ± SD pulmonary $\dot{V}O_2$ and heart rate measured during 2 h of heavy-intensity cycle exercise. Note the increase in relative exercise intensity over time. a = different from 10-15 min (P<0.05), b = different from 25-30 min (P<0.05), c = different from 55-60 min (P< 0.05), d = different from 85-90 min (P<0.05).*

The standard error (and CV%) for the Fatigued-CP parameter estimate from the 'best fit' model was 3 ± 3 W (1.1 ± 1.1%), and the standard error (and CV%) for Fatigued-W' for the 'best fit' model was 1.3 ± 1.2 kJ (8.9 ± 9.0%). The 'best fit' model was provided by the 1/time model for 7 subjects and by the hyperbolic model for the other 7 subjects.

Fatigued-EP (256 ± 52 W) and Fatigued-CP (256 ± 41 W) were not different from one another (95% confidence limits 13, -13 W; $P = 0.94$) but were ~11% lower than Control-EP (287 ± 46 W; $P<0.005$; Fig. 2A). The intra-class correlation coefficient for Fatigued-CP and Fatigued-EP was $r = 0.91$ ($P<0.001$), and the standard error of estimate was 17 W (7%) (Fig. 3A, B). Fatigued-WEP (14.6 ± 5.3 kJ) and Fatigued-W' (15.3 ± 5.0 kJ) were not different from one another (95% confidence limits 3.6, -2.3 kJ; $P=0.65$) but were 22% and 17% lower, respectively, compared to Control-WEP (18.7 ± 4.7 kJ; $P<0.05$; Fig. 2B). The intra-class correlation coefficient for Fatigued-WEP and Fatigued-W' was $r = 0.52$ ($P = 0.59$), and the standard error of estimate was 4.4 kJ (29%) (Fig. 3C, D). The changes in EP and WEP observed over the 2-h exercise bout were not significantly correlated ($r = -0.18$; $P=0.54$). The peak power output was not different between the Fatigued-3MT (1083 ± 246 W) and the Control-3MT (1037 ± 389 W; $P=0.48$). Total work done was ~14% lower during the Fatigued-3MT (60.7 ± 12.6 kJ) compared to the Control-3MT (70.2 ± 9.6 kJ; $P<0.001$).

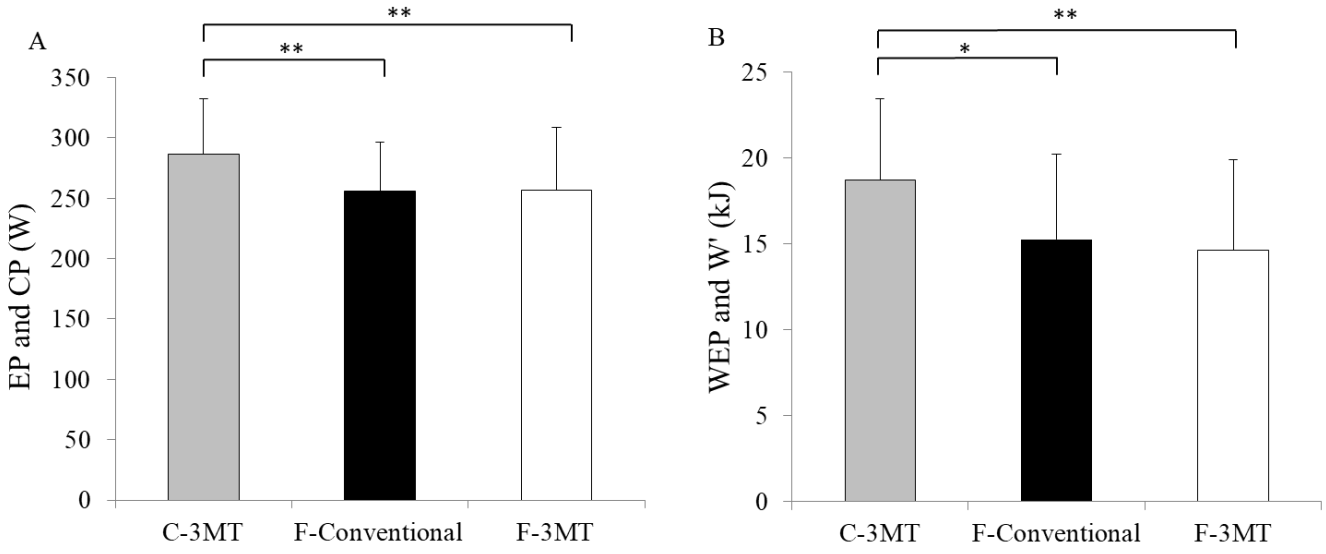


***Figure 2.*** *Panel A: End-test power (EP) in a rested state (Control-3MT) and Fatigued state (Fatigued-3MT), and critical power (CP) derived from a conventional prediction trial protocol in a Fatigued state (F-Conventional). Panel B: Group mean work done above end-test power (WEP) in a rested state (Control-3MT) and Fatigued state (Fatigued-3MT), and W' derived from a conventional prediction trial protocol in a Fatigued state (F-Conventional). * = P<0.01, ** = P<0.05.*

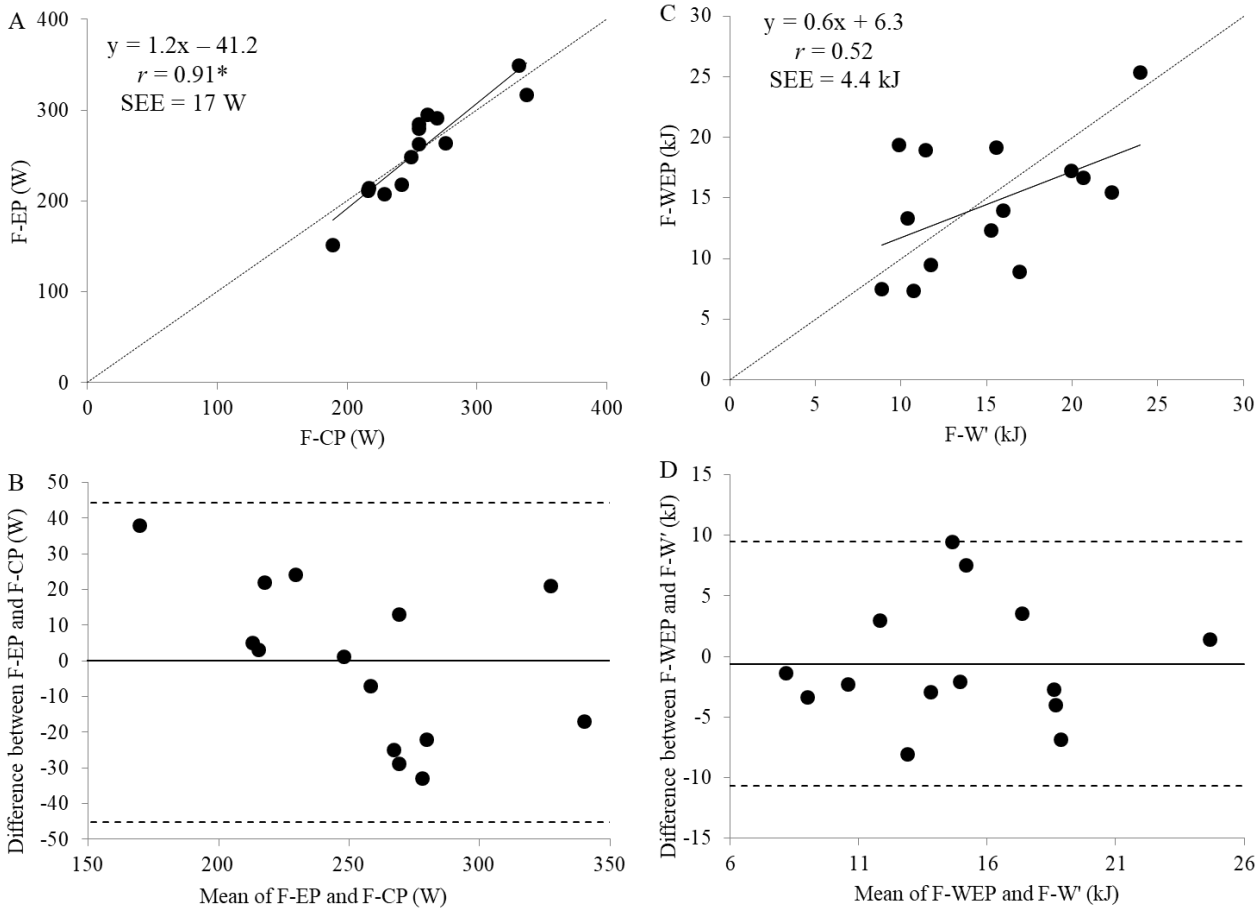


***Figure 3.*** *Bland-Altman plots of the relationship and limits of agreement between end-test power (Fatigued-EP) and critical power (Fatigued-CP) (panels A and B), and work done above EP (Fatigued-WEP) and W′ (Fatigued-W′) (panels C and D) after 2 h of heavy-intensity exercise. Fatigued-EP and Fatigued-WEP were estimated using a 3-min all-out test and the CP and W′ were derived from a conventional prediction trial protocol. In panels A and C the solid line is the best-fit linear regression and the dashed line is the line of identity. In panels B and D the solid horizontal line represents the mean difference between the two measurements and the dashed lines represent limits of agreement. * = P<0.01.*

Muscle [glycogen] decreased by ~65% over the 2-h heavy-intensity exercise bout (Pre: 639 ± 235 mmol/kg d.w, Post: 226 ± 194 mmol/kg d.w; Fig. 4A; $P<0.001$). There was no significant correlation between the decline in muscle [glycogen] (413 ± 116 mmol/kg d.w) and the difference between Control-EP and Fatigued-EP (30 ± 27 W; $r = 0.19$; $P=0.52$). Moreover, the decline in muscle [glycogen] was not significantly correlated with the difference between Control-WEP and Fatigued-WEP (4.1 ± 3.3 kJ; $r = 0.07$; $P=0.80$).

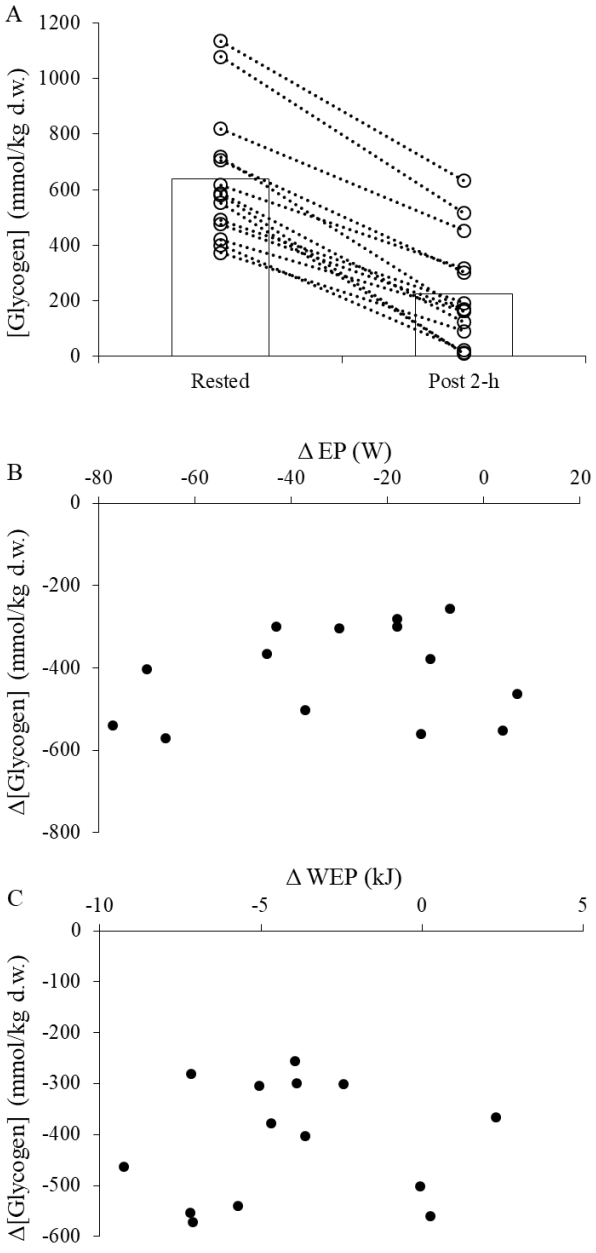


***Figure 4.*** *Muscle [glycogen] before and after 2 h of heavy-intensity exercise (panel A). There were no significant correlations between the change (Δ) in muscle [glycogen] and changes in EP (ΔEP; panel B) or WEP (ΔWEP; panel C) estimated in a 3-min all-out test at rest and after 2 h of heavy-intensity exercise. Dashed lines in panel A represent individual responses.*

The constant power outputs for the short, intermediate and long severe-intensity prediction trials and the <Fatigued-CP test were 336 ± 60 W, 302 ± 52 W, 281 ± 46 W and 241 ± 41 W, respectively. The $T_{lim}$ for the short (199 ± 55 s), intermediate (362 ± 92 s) and long (668 ± 119 s) severe-intensity prediction trials were within the desired range. Five (of 14) participants were able to complete the target of 30 min during the <Fatigued-CP test; the $T_{lim}$ for the remaining 9 participants was 1193 ± 295 s. There was an increase in $\dot{V}O_2$ from the end of the 2-h heavy-intensity exercise bout to the end of all the severe-intensity prediction trials as well as to the end of the <Fatigued-CP bout ($P$<0.001; Fig. 5). There were no differences in $\dot{V}O_{2peak}$ measured in the ramp incremental test (4.31 ± 0.35 L·min$^{-1}$) and the short (4.37 ± 0.41 L·min$^{-1}$), intermediate (4.32 ± 0.31 L·min$^{-1}$) and long (4.36 ± 0.38 L·min$^{-1}$) severe-intensity prediction trials. $\dot{V}O_{2peak}$ in the <Fatigued-CP test (3.99 ± 0.45 L·min$^{-1}$) was lower than $\dot{V}O_{2peak}$ during the ramp incremental test and the short, intermediate and long prediction trials ($P$<0.05; Fig. 5). There were no differences in $\dot{V}O_{2peak}$ between the Control-3MT (4.32 ± 0.32 L·min$^{-1}$), the Fatigued-3MT (4.42 ± 0.30 L·min$^{-1}$) and the ramp incremental test. $HR_{max}$ obtained during the ramp incremental test (178 ± 8 b·min$^{-1}$) was not different from end-exercise HR in the short (178 ± 10 b·min$^{-1}$), intermediate (178 ± 9 b·min$^{-1}$) and long (177 ± 10 b·min$^{-1}$) prediction trials or the <Fatigued-CP test (171 ± 13 b·min$^{-1}$).

Blood [lactate] increased from the end of the 2-h heavy-intensity exercise bout to $T_{lim}$ in all four subsequent exercise tests ($P$<0.005). End-exercise blood [lactate] was lower ($P$<0.05) during the <Fatigued-CP exercise test (3.8 ± 2.7 mM) compared to the short (5.6 ± 1.8 mM), intermediate (6.4 ± 3.1 mM) and long (6.4 ± 2.9 mM) severe-intensity prediction trials but was not different between the three severe-intensity prediction trials.

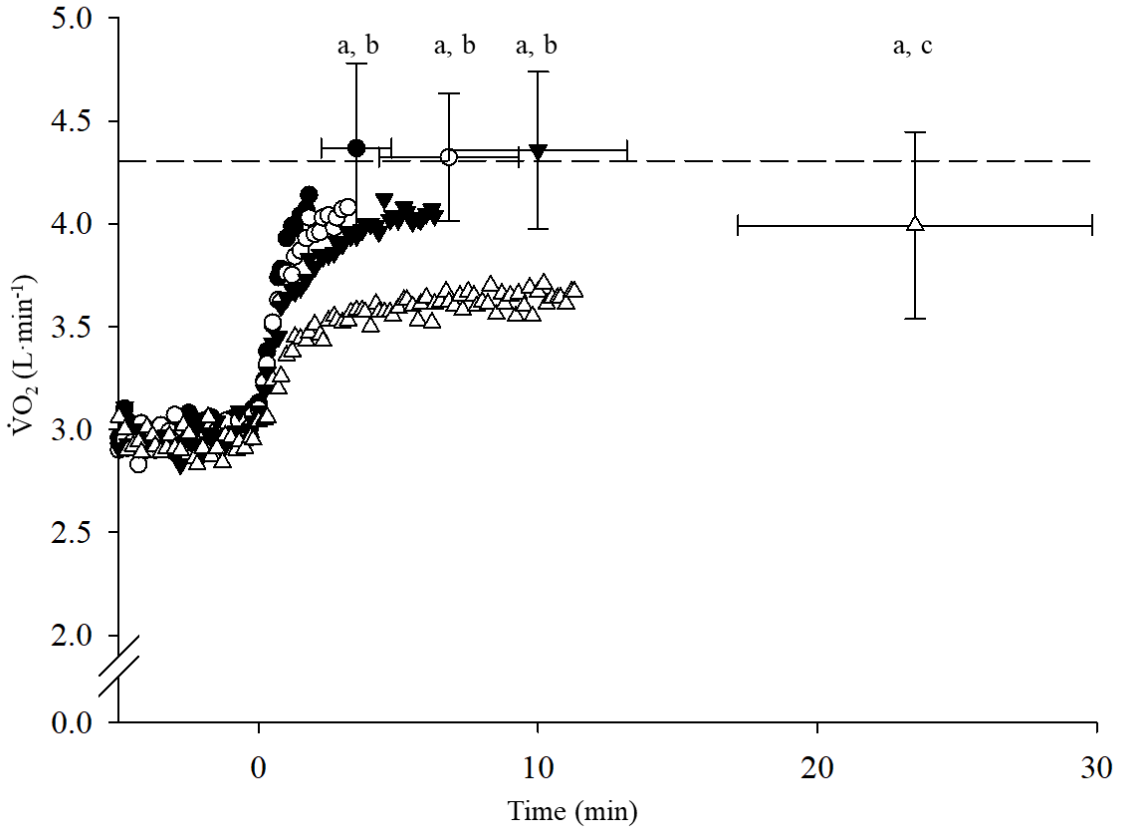


***Figure 5.*** *Pulmonary $\dot{V}O_2$ during the short (black circles), intermediate (white circles) and long (black triangles) severe-intensity prediction trials and the <Fatigued-CP trial (white triangles). The dashed horizontal line indicates $\dot{V}O_{2peak}$ measured in the ramp incremental test. Error bars (SD) are shown for end-exercise time points only to aid clarity. a = different from $\dot{V}O_2$ measured at the end of 2 h of heavy-intensity exercise (P<0.01); b = different from end-exercise $\dot{V}O_2$ measured in the <Fatigued-CP trial (P<0.01); c = different from $\dot{V}O_2$ measured at ~10 min in the <Fatigued-CP trial (P<0.01).*

## 6.4 Discussion

This is the first study to investigate changes in the parameters of the power-duration relationship (CP and W') after prolonged endurance exercise estimated using both the conventional protocol and the 3MT. Consistent with our experimental hypotheses, following 2 h of heavy-intensity exercise: 1) the Fatigued-CP and Fatigued-W' measured using the conventional protocol were significantly lower (by 11% and 20%, respectively) compared to the values estimated in the absence of prior exercise; and 2) the Fatigued-EP measured using the Fatigued-3MT provided an accurate (SEE 7%) estimate of the Fatigued-CP established using the conventional protocol, while the agreement between Fatigued-WEP and Fatigued-W' was limited (SEE 29%). However, contrary to our third hypothesis, there were no significant correlations between muscle glycogen depletion and the change in either EP or WEP following 2 h of heavy-intensity exercise. The results of this study provide evidence that the parameters of the power-duration relationship are profoundly altered by prolonged endurance exercise, with implications for the prediction of performance during such exercise based on parameters measured in a rested state. Understanding dynamic changes in these parameters may provide insight into the nature of fatigue development during such exercise and enable the development of interventions to enhance human performance.

The EP and WEP declined when estimated following 2 h of heavy-intensity exercise compared to a rested state. Compared to Control-EP, there was a 10% reduction in Fatigued-CP and an 11% reduction in Fatigued-EP with no significant difference between Fatigued-CP and Fatigued-EP. Similarly, compared to Control-WEP, there was a 17% reduction in Fatigued-W′ and a 22% reduction in Fatigued-WEP. There was no

significant difference between Fatigued-W′ and Fatigued-WEP, but it is important to note that Fatigued-W′ and Fatigued-WEP were not significantly correlated and showed limited agreement with an SEE of 4.4 kJ or 29%. These findings indicate, for the first time, that the CP parameter of the power-duration relationship estimated with the 3MT was not different from that estimated using the conventional protocol after 2 h of heavy-intensity exercise, and confirm our previous findings that such exercise leads to substantial reductions in CP and W′ of ~10% and ~20%, respectively (8). We previously reported that the power profile during the Fatigued-3MT was highly reproducible (8). In the present study, the close agreement between Fatigued-EP and Fatigued-CP following 2 h of heavy-intensity exercise provides confidence in the sensitivity and practicality of the Fatigued-3MT to accurately evaluate changes in CP during prolonged, fatiguing, endurance exercise. It should be noted, however, that the Fatigued-3MT provided a much more accurate estimate of Fatigued-CP (7% error) than of Fatigued-W′ (29%). This observation is consistent with greater test-retest variability of the WEP and W' compared to EP and CP, respectively, in the rested state (37, 38). It is important to recognise that, when determined using conventional procedures, the test-retest SEE for W′ (~14%) is generally higher than for CP (~4-8%), (12, 27).

The relative intensity over the 2 h of heavy-intensity exercise increased from ~65 to ~72% $\dot{V}O_{2peak}$ which is in accordance with our previous findings (8). This 'drift' in $\dot{V}O_2$, which is mechanistically distinct from the $\dot{V}O_2$ slow component (22), reflects, in part, the reduction in RER due to increased reliance on fat compared to carbohydrate oxidation. Alongside this, in the present study we found that muscle [glycogen] was reduced by ~65% during the 2-h exercise bout. When muscle [glycogen] reaches low values, the

reliance on fat oxidation is increased to sustain exercise, especially when carbohydrate supplements are not provided (9), and exercise performance is typically impaired (17). However, we found no significant correlations between muscle glycogen depletion during the 2-h heavy-intensity exercise bout and the decrease in EP or WEP. Our findings therefore suggest that muscle glycogen depletion did not occur in parallel with changes in the parameters of the power-duration relationship following 2 h of heavy-intensity exercise. It should be noted, however, that the relationship between absolute muscle [glycogen], measured at a discrete site in the *m. vastus lateralis*, and the rate of energy supply from carbohydrate to support whole-body oxidative metabolism (i.e., CP) is unclear and may not be directly proportional.

The physiological basis for the changes observed in EP after 2 h of heavy-intensity exercise is likely multifactorial. Peripheral factors, such as changes in high-energy phosphates and pH, would seem to be unlikely candidates given that exercise of similar duration and intensity does not appreciably perturb the intramuscular milieu (4, 33) and blood [lactate] remained low and stable across time in the present study. An alteration in neuromuscular excitability would also seem an unlikely explanation (28) given that plasma [$K^+$] was stable over the final 90 min of the 2-h heavy-intensity exercise bout. Acute changes in mitochondrial function, such as increased uncoupling, during endurance exercise would reduce power output for a given $\dot{V}O_2$ and could explain a lower EP. However, while the expression of uncoupling protein 3 has been reported to be increased in rat skeletal muscle following 2-h of endurance exercise (20), similar effects have not been consistently demonstrated in humans (10, 35). It is known that critical torque (the analogue of CP) measured during knee extension exercise

represents a critical threshold for neuromuscular fatigue development (6) such that so-called central fatigue makes a greater contribution to fatigue development and exercise intolerance in the heavy-intensity domain compared to the severe-intensity domain (4, 6). Consistent with this, Thomas et al. (34) reported a greater degree of central fatigue, as determined by greater reductions in voluntary activation measured by motor nerve and cortical stimulation, during self-paced cycle exercise requiring >30 min duration compared to shorter exercise bouts. It is possible, therefore, that the development of central fatigue during the 2-h heavy-intensity exercise bout in the present study influenced the subsequent severe-intensity prediction trials and the 3MT, limiting exercise performance and reducing CP and EP.

Other possible contributory factors to the reduction in EP following 2-h heavy-intensity exercise include the development of muscle damage, respiratory muscle fatigue, and challenges to thermoregulation. The submaximal cycling exercise performed in the present study has no eccentric component and is therefore unlikely to result in significant muscle damage (31). It is possible, however, that muscle damage incurred during prolonged exercise in other modalities which have a greater eccentric muscle action, such as running, results in greater changes in the speed-time relationship than we report herein for cycling. While respiratory muscle fatigue can develop during prolonged endurance exercise, despite relatively low rates of ventilation, effects on performance are controversial and unlikely to be appreciable (15). We did not measure core temperature or sweat rate in the present study but participants were allowed to consume water *ad libitum* such that the reduction in body mass over the prolonged exercise bout was constrained to ~0.9 kg. In more extreme environmental conditions

(high heat and/or humidity, or indeed at altitude), or when opportunities for fluid replacement are limited, it is possible that the deleterious effects of prolonged exercise on the power-time relationship may be amplified.

We asked participants to complete an exercise bout at 15 W below Fatigued-CP to test the assumption that exercise performed <Fatigued-CP would produce physiological responses consistent with exercise in the heavy-intensity domain, as is the case when CP is determined without prior fatiguing exercise (4, 5, 32). We found that exercise <Fatigued-CP could not be sustained for 30 min by all participants following 2 h of heavy-intensity cycling. Indeed, only five out of the 14 participants were able to complete 30 min of exercise <Fatigued-CP. The remaining nine participants were unable to complete 30 min of exercise <Fatigued-EP despite steady-state $\dot{V}O_2$ and blood [lactate] profiles being evident in eight of them. Given that the participants displayed physiological responses which were indicative of heavy-intensity exercise in the <Fatigued-CP test, it may be considered surprising that the majority of them could not complete 30 min of exercise. However, this is likely the result of muscle glycogen depletion. Muscle [glycogen] was decreased in all participants during the 2-h exercise bout, albeit with substantial inter-subject variability. Interestingly, muscle [glycogen] was 37 ± 46 mmol/kg d.w. after the 2-h heavy-intensity exercise bout in participants who reached $T_{lim}$ in <20 min in the <Fatigued-CP test, compared to 277 ± 187 mmol/kg d.w. in the participants who completed >20 min of exercise. Moreover, the participants who completed <20 min exercise in the <Fatigued-CP test exhibited a larger decrease in CP (59 ± 16 W) than participants who completed >20 min exercise (20 ± 22 W). It might be speculated that a low muscle [glycogen] at the start of the <Fatigued-CP test restricted

carbohydrate supply and 'rate-limited' oxidative metabolism such that the external power output could not be maintained.

Two hours of heavy-intensity exercise resulted in a ~20% reduction in WEP, but this was not correlated with the fall in muscle [glycogen]. This result is perhaps surprising given that Miura et al. (24) reported a ~20% reduction in W', with no change in CP, following an exercise and dietary regimen designed to result in muscle glycogen depletion. During long-duration endurance exercise there is a decrease in muscle [glycogen] in both type I and type II muscle fibres (13, 14). A low muscle [glycogen] impairs sarcoplasmic reticulum $Ca^{2+}$ release, leading to excitation-contraction coupling failure and reduced force production (7). Considering the results of the present study alongside those of Miura et al. (24), it appears that glycogen depletion either limits energy production above CP or results in earlier/greater accumulation of metabolites for a given amount of work done above CP, with total work capacity being reduced in either case. Despite the lack of significant correlation between changes in WEP and muscle [glycogen] in the present study, it remains possible that low muscle [glycogen] could impact WEP and W', albeit in a more complex fashion (8, 24). It is interesting to note here that completing severe-intensity or sprint exercise immediately prior to a 3MT reduces WEP and peak power output without affecting EP (30, 39) whereas completing heavy-intensity exercise reduces WEP and EP but not peak power output (present study). This dissimilarity is presumably related to differential effects of these prior exercise protocols on muscle [PCr] and [glycogen]. It is also possible that muscle [glycogen] influences WEP and W′ differently due to differences in motor unit

recruitment patterns evident in the 'all-out' 3MT compared to the constant-power, severe-intensity prediction trials employed in the conventional protocol (38, 41).

## Experimental Considerations

To reduce the demand on the participants, which was already significant, we did not measure CP and W′ using conventional severe-intensity prediction trials when the subjects had not completed preceding exercise but rather relied on the 3MT to estimate these parameters. However, it is well established that the EP and WEP measured in a 3MT provides valid and reliable estimates of CP and W′ in moderately-trained subjects, provided that the test is performed against appropriately normalized fixed resistance and the $\dot{V}O_{2max}$ is attained and maintained (22, 29, 37, 38; cf. 26). A possible limitation of our study was that pre- and post-2 h exercise muscle biopsies were only obtained on one of the visits. However, participants kept a food and training diary and replicated their dietary and physical activity before each visit in order to minimize the likelihood of large differences in pre-exercise muscle [glycogen] between tests. Baseline, end-exercise and changes in HR, body mass and blood [lactate] were similar in all of the 2-h exercise bouts, providing reassurance that the physiological demands of the repeated 2-h exercise bouts were consistent. Another limitation was that the relatively large amount of tissue required to measure [glycogen] precluded the measurement of other intramuscular substrates and metabolites (e.g. PCr, lactate), although these would not be expected to change substantially (4). Finally, it should be acknowledged that muscle biopsy samples are obtained from a small area of the active muscle mass engaged during cycle exercise such that the lack of correlation between individual changes in muscle [glycogen] and changes in EP and WEP does not exclude the possibility that

muscle glycogen availability makes an important contribution to changes in the parameters of the power-duration relationship reported in the present study.

## Perspectives and Significance

The power-duration relationship has significant utility in predicting performance and optimizing athletic training programs (21, 40). However, the results of the present study indicate that the values of both CP and W′ are subject to change during and following prolonged endurance exercise. These findings have important implications for the prediction of sporting performance and for optimal pacing strategy. Dynamic changes in the parameters of the power-duration relationship during fatiguing exercise could mean that a given speed/power output predicted to reside within the heavy-intensity exercise domain may, at some stage during competition, begin to elicit physiological responses characteristic of the severe-intensity domain. Performance in endurance competition therefore depends not only upon the CP and W′ measured in a 'fresh' state but also on the extent to which these parameters deteriorate during fatiguing exercise. Further research is necessary to investigate the extent to which CP and W' are affected by fatigue development in other exercise settings, the time course over which CP and W′ decline during prolonged exercise, and the efficacy of various interventions to offset these effects. The findings of the present study indicate that the 3MT may provide a practical and expeditious approach to elucidate dynamic changes in the power-duration relationship during endurance exercise.

In conclusion, the parameters of the power-duration relationship were appreciably reduced when estimated following 2 h of heavy-intensity exercise compared to the

rested state. The reductions in CP (~10%) and W′ (~20%) were similar when estimated with the conventional protocol and the 3MT, indicating that the 3MT may be used to conveniently estimate the CP and W′ under these conditions. The changes in EP and WEP following 2 h of heavy-intensity exercise were not significantly correlated with the reduction in muscle [glycogen]. Importantly, when CP is estimated in a Fatigued condition, subsequent exercise performed ostensibly below CP cannot be sustained beyond ~20 min despite the attainment of a physiological steady-state. This indicates that the 'characteristic' physiological responses elicited during <CP exercise differ when assessed following prolonged endurance exercise, an effect that may be related to low pre-test muscle [glycogen]. These results may have important implications for understanding the interaction between fatigue development and performance capacity during prolonged endurance exercise.

**Acknowledgements**

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## Summary of chapter 6

Chapter 6 showed that following 2 h of heavy-intensity exercise, CP and W′ estimated using the conventional method did not differ from EP and WEP estimated using the 3MT. This indicates that the 3MT can be used to predict accurate estimates of CP and W′ following a period of prolonged heavy-intensity exercise. Additionally, chapter 6 indicated that the reduction in CP and W′ following 2 h of heavy-intensity exercise was not explained solely by muscle glycogen depleted. In light of these findings, the purpose of chapter 7 was to identify the time-course of changes in EP and WEP and to investigate whether carbohydrate ingestion would attenuate the reduction in EP and WEP following 2 h of heavy-intensity exercise.

# Chapter 7. Dynamics of the power-duration relationship during prolonged endurance exercise and influence of carbohydrate ingestion

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## Abstract

We tested the hypotheses that the parameters of the power-duration relationship, estimated as the end-test power (EP) and work done above EP (WEP) during a 3-min all out exercise test (3MT), would be reduced progressively following 40 min, 80 min and 2 h of heavy-intensity cycling, and that carbohydrate (CHO) ingestion would attenuate the reduction in EP and WEP. Sixteen participants completed a 3MT without prior exercise (control), immediately after 40 min, 80 min and 2-h of heavy-intensity exercise while consuming a placebo beverage, and also after 2-h of heavy-intensity exercise while consuming a CHO supplement (60 g/h CHO). There was no difference in EP measured without prior exercise (260 ± 37 W) compared to EP following 40 min (268 ± 39 W) or 80 min (260 ± 40 W) of heavy-intensity exercise; however, after 2-h, EP was 9% lower compared to control (236 ± 47 W; $P<0.05$). There was no difference in WEP measured without prior exercise (17.9 ± 3.3 kJ) compared to after 40 min of heavy-intensity exercise (16.1 ± 3.3 kJ), but WEP was lower ($P<0.05$) than control after 80 min (14.7 ± 2.9 kJ) and 2-h (13.8 ± 2.7 kJ). Compared to placebo, CHO ingestion negated the reduction of EP following 2-h of heavy-intensity exercise (254 ± 49 W) but had no effect on WEP (13.5 ± 3.4 kJ). These results reveal a different time course for the deterioration of EP and WEP during prolonged endurance exercise and indicate that EP is sensitive to CHO availability.

## New and Noteworthy

The parameters of the power-duration relationship (critical power, CP, and the curvature constant, W') have typically been considered to be static. Herein, we report the time course for reductions in CP and W', as estimated using the 3-min all-out cycle test, during 2 h of heavy-intensity exercise. We also show that carbohydrate ingestion during exercise preserves CP, but not W', without altering muscle glycogen depletion. These results provide new mechanistic and practical insight into the power-duration curve and its relationship to exercise-related fatigue development.

## 7.1 Introduction

The parameters of the hyperbolic power-duration relationship, the critical power (CP) and the curvature constant (W′, which represents a fixed work capacity above CP), are important determinants of endurance exercise performance (25, 32, 50). CP is considered to be a metabolic or fatigue threshold which separates the 'heavy' from the 'severe' exercise intensity domains (8, 40, 41). In the heavy-intensity domain (<CP), intramuscular metabolic homeostasis is maintained and pulmonary $\dot{V}O_2$ attains a delayed steady-state (4, 26, 41). In contrast, in the severe-intensity domain (>CP), intramuscular metabolic homeostasis is not achieved, a $\dot{V}O_2$ 'slow component' develops that drives $\dot{V}O_2$ inexorably to its maximum, and exercise tolerance is predictably limited as a function of the power output above CP and the size of the W' (4, 26, 40, 41).

The CP and W′ are conventionally estimated using 3-5 severe-intensity prediction trials in which constant power outputs are maintained until the limit of tolerance, with the asymptote (representing CP) and the curvature constant (representing W′) of the power-time relationship subsequently being determined mathematically (30, 41, 48). More recently, a 3-min all-out cycle test against fixed resistance (3MT) has been developed, during which external power output declines hyperbolically with time, which permits a more expeditious assessment of CP and W′ (7, 48). The 3MT has been shown to provide valid and reliable estimates of CP and W', where the mean power output during the last 30 s of the test (end-test power, EP) represents CP, and the work completed above EP (WEP) represents W' (7, 38, 48). We have recently reported that the 3MT continues to provide valid (13) and reasonably reliable (12) estimates of CP and W′

following 2 h of heavy-intensity cycle exercise. In these studies we found that prolonged endurance exercise consistently and profoundly altered the power-time relationship, with CP and EP falling by ~10% and W′ and WEP falling by ~20% (12, 13). These results have important implications for our understanding of fatigue development, and for performance prognosis, during endurance exercise. At present, however, the time course over which EP and WEP deteriorate during prolonged endurance exercise is not known. Elucidating the dynamic changes in EP and WEP *during* prolonged exercise may provide insight into the determinants of fatigue and underpin the development of interventions to attenuate the decline in performance during such exercise.

The mechanistic bases for the reductions in EP and WEP after 2 h of heavy-intensity exercise are likely multifactorial, and may include muscle glycogen depletion (12, 13, 30). It is well known that glycogen depletion increases with the duration of heavy-intensity exercise (17, 18). If glycogen depletion impacts the power-duration relationship, then reductions in EP and WEP would be expected to become much more substantial following longer, compared to shorter, bouts of heavy-intensity exercise. Carbohydrate (CHO) ingestion is known to benefit prolonged endurance exercise performance by sparing muscle [glycogen], better maintaining blood [glucose] and/or providing stimulation to the central nervous system via the ‘pleasure and reward’ centers of the brain (21, 23). During long-duration events, which deplete muscle glycogen stores, exogenous CHO intake is particularly important to maintain high rates of CHO oxidation (20), with the greatest performance enhancement observed at ingestion rates of 60-80 g/h (44). While the reductions in EP and WEP were not

significantly correlated with changes in muscle [glycogen] following 2 h of heavy-exercise in our previous study (13), the relationship between muscle CHO availability (in the muscle and circulation) and the power-time relationship is likely to be complex, and it is possible that CHO ingestion may offset the reductions in EP and WEP reported following 2 h of heavy-intensity cycling (12, 13)

The purpose of this study was to: 1) investigate the dynamic changes in EP and WEP during and following 2 h of heavy-intensity exercise; and 2) determine the effect of 60 g/h of CHO ingestion, compared to a placebo, on EP and WEP during and following 2 h of heavy-intensity exercise. We hypothesized that EP and WEP would be reduced progressively following 40 min, 80 min and 2 h of heavy-intensity exercise, and that CHO ingestion would attenuate the declines in EP and WEP after 2 h of heavy-intensity exercise.

## 7.2 Methods

This paper reports the results of two experiments. The first experiment was conducted to investigate possible changes in EP and WEP after 40 min, 80 min and 2 h of heavy-intensity exercise and the second experiment was conducted to investigate the effect of CHO ingestion on changes in EP and WEP after 2 h of heavy-intensity exercise.

*Participants*

Experiments I and II were conducted on the same group of participants. Sixteen males (mean ± SD: age = 34 ± 6 years, height = 1.78 ± 0.07 m, body mass = 79.1 ± 7.6 kg,

peak $O_2$ uptake ($\dot{V}O_{2peak}$) = 52.5 ± 7.3 $mL \cdot kg^{-1} \cdot min^{-1}$) took part in the experiments. They were all competitive athletes (comprising two runners, six cyclists, four triathletes, three Crossfit athletes, and one squash player) but were not professional/elite. The participants were instructed to arrive at the laboratory in a rested and hydrated state, to avoid alcoholic drinks and strenuous exercise for 24 h prior to testing, and to maintain their habitual diet throughout the study. The participants recorded their diet for 24 h prior to the first experimental session and replicated this prior to each subsequent visit. The experimental procedures were approved by the Institutional Research Ethics Committee at the University of Exeter and informed consent was obtained from each participant prior to testing. One participant did not consent to having muscle biopsies taken in Experiment II. All exercise tests were separated by a minimum of 24 h but the tests in which the 3MT was completed following a bout of heavy-intensity exercise (see below for more details) were separated by at least 72 h (i.e., 7 ± 4 days).

*Experimental design*

Participants reported to the laboratory on 7 occasions over a 7-week period (± 2 weeks). The tests included a ramp incremental exercise test for the determination of $\dot{V}O_{2peak}$ and gas exchange threshold (GET), a 3MT familiarisation trial, a 3MT performed in a rested state which served as a control (C-3MT), and on subsequent visits: a 3MT preceded by 40 min (40-3MT), 80 min (80-3MT) or, on two occasions, 2 h of heavy-intensity exercise. A placebo drink was consumed during the 40-3MT, 80-3MT and during one of the 2 h visits (120-$3MT_{PLA}$), while CHO was consumed during one 2 h visit

(120-3MT$_{CHO}$). The 120-3MT$_{PLA}$ visit was used for both experiments. The other trials were administered in a randomized and counterbalanced order.

All exercise tests were conducted using the same electrically-braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). During all tests, except the 3MTs, the participants cycled at a self-selected pedal rate (70-90 rpm). For each participant, the self-selected pedal rate used in the ramp incremental test was recorded and replicated in each subsequent visit. The ergometer seat and handlebar configuration were adjusted for comfort during the first visit and were recorded and then replicated in subsequent visits. Exercise tests took place in an air-conditioned laboratory with ambient temperature of 20$^{o}$C and relative humidity of 60%.

*Determination of $\dot{V}O_{2peak}$ and gas exchange threshold*

During visit 1 the participants completed a ramp incremental exercise test. The ramp protocol consisted of a 3-min baseline of pedalling at 20 W after which the power output was increased by 30 W/min until the participant was unable to continue. The limit of tolerance was determined when cadence fell >10 rpm below the target cadence for more than 5 s despite strong verbal encouragement. $\dot{V}O_{2peak}$ was determined as the highest 30-s mean value recorded during the test. The GET was defined according to the procedures of Beaver et al. (2). The GET and $\dot{V}O_{2peak}$ were used to normalize the fixed resistance for the 3MTs.

*Three-min all-out tests (3MTs)*

The resistance for the 3MT was applied using the linear factor function of the ergometer and was calculated as: linear factor = power output/preferred cadence$^2$ where the power was 50%Δ (i.e., GET plus 50% of the interval between the GET and ramp test peak power output). Visit 2 (familiarization test) and visit 3 consisted of a C-3MT performed with no prior exercise. The 3MT protocol began with a 3-min baseline of pedalling at 20 W. During visits 4-7, a 60 s pause was administered after the constant power output bout, during which the participants had 30 s of passive recovery and then were instructed to cycle for the last 30 s against a resistance of 20 W. This was done to replicate the 1-min pause that was required to obtain a muscle biopsy in Experiment II. A 5 s countdown was given prior to the 3MT and the participant was instructed to increase cadence to ~110-120 rpm. Strong verbal encouragement was then given for an all-out effort from the onset of the 3MT. Participants were not informed of the elapsed time. Instructions were given to reach peak power output as quickly as possible and to maintain the all-out effort throughout the test. The EP was subsequently calculated as the mean power output over the last 30 s of the test, and the WEP was calculated as the work done above EP during the 3MT, i.e. the mean power output above EP for each second of the test was multipled by the number of seconds of exercise performed above EP to compute the total work done above EP (48). The results of the 3MT were deemed valid only when the $\dot{V}O_{2peak}$ attained exceeded 95% of the $\dot{V}O_{2peak}$ determined in the initial ramp incremental test. Results determined from the C-3MT, 40-3MT, 80-3MT, 120-3MT$_{PLA}$ and 120-3MT$_{CHO}$ were consequently termed C-EP and C-WEP, 40-EP and 40-WEP, 80-EP and 80-WEP, 120$_{PLA}$-EP and 120$_{PLA}$-WEP, 120$_{CHO}$-EP and 120$_{CHO}$-WEP, respectively.

*Experiment I: Effect of 40 min, 80 min and 2 h of heavy-intensity exercise on the parameters of the power-duration relationship*

The constant power output applied during the 40 min, 80 min and 2 h heavy-intensity exercise bouts was calculated as the power output at the GET plus 25% of the difference between the GET and C-EP (25%Δ1; 12). Experiment I consisted of three heavy-intensity exercise bouts of 40 min, 80 min and 2 h followed by a 3MT. The visits began with a 3-min baseline of pedaling at 20 W for attainment of baseline measurements, after which the power output abruptly increased to the target power output for 40 min, 80 min or 2 h. Participants were instructed to hold their desired cadence throughout the constant power output bout. Participants were provided with a 100-ml opaque plastic bottle containing 1 mL of apple-flavored sweetener (Myprotein.Co, UK), with no caloric value, added to 94 mL of water to ensure that supplements were indistinguishable from, and of equal volume to, the CHO solution used in Experiment II (see below). The first bottle was given at -3 min, as participants wore a mask between -3 – 15 min for measurements of pulmonary gas exchange, and thereafter a bottle was given every 15 min. During the 40-3MT the last beverage was consumed at 30 min; during the 80-3MT visit the last beverage was consumed at 75 min; and during the 120-3MT$_{PLA}$ the last beverage was consumed at 105 min. A clock with time remaining was visible during the constant power output bout and participants were allowed to listen to music; however, both were withdrawn 2 min prior to the 3MT. Participants were instructed to stop pedalling for 30 s after the constant power output bout, and at 30 s, they were instructed to start cycling again at 20 W. This was

administered to ensure the same rest period was provided between the four constant power output tests (both experiments) and the 3MTs and so that muscle biopsies could be taken in the 30 s window during the 2 h visits for experiment II. Pulmonary gas exchange data were attained at the following time points during the 40-3MT visit: -3-15, 25-30 and 35-40 min; the 80-3MT visit: -3-15, 25-30, 55-60 and 75-80 min; and the 120-$3MT_{PLA}$ visit: -3-15, 25-30, 55-60, 85-90 and 115-120 min; and continuously throughout the 3MT. A blood sample from a fingertip was collected every 20 min during the constant power output bouts for the analysis of [lactate] and [glucose]. Heart rate (HR) was recorded (Garmin FR70, Garmin Ltd, Schaffhausen, Switzerland) every 5 s during all visits. Before and after the test, participants were weighed in minimal clothing to assess changes in body mass.

*Experiment II: Effect of CHO ingestion on the parameters of the power-duration relationship*

The 120-$3MT_{PLA}$ and 120-$3MT_{CHO}$ visits included 2 h of heavy-intensity exercise immediately followed by a 3MT. The 120-$3MT_{PLA}$ exercise trial was the same as that in Experiment I. The procedures for the 120-$3MT_{CHO}$ visit were identical to the 2 h visit described in Experiment I, except for the ingestion of CHO. During the 120-$3MT_{CHO}$ visit participants consumed 60g/h of CHO (Maurten drink mix 320, Biotech center, Gothenburg, Sweden). Participants were given the same bottle as described in Experiment I, containing 94 ml of Maurten drink mix (15g of CHO), every 15 min during the constant power output bout. A muscle biopsy was taken prior to exercise and again

at 120 min during the 30 s rest period between the constant-power-output bout and the 3MT.

*Measurements*

**Pulmonary gas exchange.** Pulmonary gas exchange was measured breath-by-breath and averaged over 10 s periods during all visits. Participants wore a face mask (Hans Rudolf 7450 Series V2$^{TM}$ Mask, CareFusion, Germany) and inspired and expired gas volume and gas concentration signals were sampled continuously at 100 Hz (Vyntus, CareFusion, Germany) via a capillary line connected to the mask. These analysers were calibrated before each test with gases of known concentration and the turbine volume transducer was calibrated using a 3-L syringe (Hans Rudolph, MO). The volume and concentration signals were time-aligned by accounting for the delay in capillary gas transit and analyzer rise time relative to the volume signal. The baseline $\dot{V}O_2$ period during all visits were defined as the mean value recorded over the final minute during the 3-min warm up period at 20 W. Fat and CHO oxidation rates were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ using the following stoichiometric equations with the assumption that protein oxidation during exercise did not change (24):

CHO oxidation ($g \cdot min^{-1}$) = [4.21 ($\dot{V}CO_2$) – 2.692 ($\dot{V}O_2$)]

Fat oxidation ($g \cdot min^{-1}$) = [1.695 ($\dot{V}O_2$) – 1.701 ($\dot{V}CO_2$)]

**Muscle biopsies.** Muscle samples were obtained from one incision from the medial region of the *m. vastus lateralis* under local anesthesia (1% lidocaine) using the percutaneous Bergström needle biopsy technique under suction (3). Muscle samples

were taken at rest and immediately post 2 h of heavy-intensity exercise during the 120-$3MT_{CHO}$ and 120-$3MT_{PLA}$ visits. The post-exercise biopsies were taken while participants remained on the cycle ergometer and were typically collected within 10 s of the completion of the exercise bout. Biopsy samples were immediately frozen in liquid nitrogen and stored at −80°C for subsequent analysis.

**Muscle glycogen concentration**. Muscle samples were freeze-dried prior to dissection from connective tissue, fat and blood. Muscle glycogen was extracted from ~1 mg d.w. muscle and hydrolysed to glucose units in 1M HCl at 95°C for 3 h. The addition of hexokinase catalyzed the reaction of glucose with adenosine triphosphate to glucose-6-phosphate, and then to 6-P-gluconolactone with NADH+ in the presence of G-6-PDH enzyme, producing the fluorescent detectable NADPH (28). Reactions were measured on a Fluoroskan (Fluoroskan™ Microplate Fluorometer, ThermoFisher Scientific, Mass. USA), with Excitation 355 nm and Emission 460 nm filters. Glycogen was reported in units of mmol of glucose per kg dry muscle.

**Blood analyses.** All fingertip blood samples (~25 µl) (visit 4-7) were collected into capillary tubes and analysed within 60 s for blood [lactate] and [glucose] using an automated lactate analyser (Stat2300, Yellow Spring Instrument, Yellow Springs, OH).

**Statistical analysis**

For experiment I, one-way ANOVAs with repeated measures were used to assess differences over time during the 40-3MT, 80-3MT and 120-$3MT_{PLA}$ tests in respiratory gas exchange variables, HR, blood [lactate] and blood [glucose]. To assess the difference in these physiological variables at common time points within the 40-3MT,

80-3MT and 120-3MT$_{PLA}$ a repeated measures ANOVA (condition x time) was used. One-way ANOVA with repeated measures was used to assess differences in EP, WEP, total work done (TWD), peak power output, $\dot{V}O_{2peak}$, and body mass between C-3MT, 40-3MT, 80-3MT and 120-3MT$_{PLA}$.

For experiment II, one-way ANOVAs with repeated measures were used to assess differences in the EP,WEP, TWD, peak power output, muscle glycogen concentration and $\dot{V}O_{2peak}$, as well as differences in respiratory gas exchange variables, blood [glucose] and blood [lactate] between C-3MT, 120-3MT$_{PLA}$ and 120-3MT$_{CHO}$. Differences in the change in muscle [glycogen] (from rest to post-exercise) between the 120-3MT$_{PLA}$ and 120-3MT$_{CHO}$ visits were analysed using a paired sample t-test. The relationships between the change in muscle [glycogen] and the changes in in EP and WEP were determined using Pearson product-moment correlation coefficients.

Statistical significance was accepted at $P<0.05$. Significant interactions and main effects were followed up with Bonferroni post hoc tests. Data are reported as mean ± SD.

## 7.3 Results

The $\dot{V}O_{2peak}$ in the ramp incremental test was 4.12 ± 0.45 L·min$^{-1}$, the peak power output was 360 ± 41 W and the GET was 132 ± 7 W. The 25%$\Delta$1 for the 2 h constant power output bouts was 164 ± 28 W.

*Experiment I: Dynamic changes in the parameters of the power-duration relationship following 40 min, 80 min and 2 h of heavy-intensity exercise*

During the 40-3MT visit, the relative intensity did not change between 10-15 min (63% ± 7% $\dot{V}O_{2peak}$) and 35-40 min (63% ± 5% $\dot{V}O_{2peak}$; $P$>0.05). The relative intensity increased during both the 80-3MT visit (from 10-15 min: 63% ± 7% $\dot{V}O_{2peak}$ to 75-80 min: 65% ± 5 % $\dot{V}O_{2peak}$; $P$<0.05) and the 120-3MT$_{PLA}$ visit (from 10-15 min: 64% ± 8% $\dot{V}O_{2peak}$ to 115-120 min: 68% ± 7% $\dot{V}O_{2peak}$; $P$<0.001). HR increased over time during all the constant power output bouts (Table 1). During the 40-3MT visit, RER did not change significantly from 10-15 min to 35-40 min; however, RER decreased during both the 80-3MT and the 120-3MT$_{PLA}$ visits (Table 1).

**Table 1.** Mean ± S.D. body mass, heart rate and RER during heavy-intensity exercise performed during various durations

| | Body mass (kg) | | Heart Rate | | RER | |
|---|---|---|---|---|---|---|
| | Pre exercise | Post exercise | 10 - 15 min | Last 5 min of exercise | 10 - 15min | Last 5 min of exercise |
| 40-3MT | 79.2 ± 7.6 | 78.9 ± 7.6[ab] | 134 ± 16 | 138 ± 16[ab] | 0.93 ± 0.03 | 0.92 ± 0.04[b] |
| 80-3MT | 79.3 ± 7.6 | 78.7 ± 7.5[ab] | 133 ± 15 | 141 ± 17[ab] | 0.93 ± 0.04 | 0.91 ± 0.05[ab] |
| 120-3MT$_{PLA}$ | 79.2 ± 7.5 | 78.2 ± 7.3[a] | 134 ± 18 | 150 ± 16[a] | 0.91 ± 0.04 | 0.84 ± 0.05[a] |
| 120-3MT$_{CHO}$ | 79.4 ± 7.6 | 78.5 ± 7.4[a] | 130 ± 16 | 149 ± 17[a] | 0.90 ± 0.05 | 0.87 ± 0.04[ab] |

40-3MT, 40 min; 80-3MT, 80 min; 120-3MT$_{PLA}$, consuming water; 120-3MT$_{CHO}$, consuming carbohydrates.
[a] different from start of exercise measurements, $P < 0.05$. [b] different from 120-3MT$_{PLA}$, $P < 0.05$.

Body mass decreased during the 40-3MT, 80-3MT and 120-3MT$_{PLA}$ trials (Table 1). Body mass was lower post-exercise in the 120-3MT$_{PLA}$ visit compared to the 80-3MT and 40-3MT visits with no differences between the 80-3MT and the 40-3MT visit (Table 1).

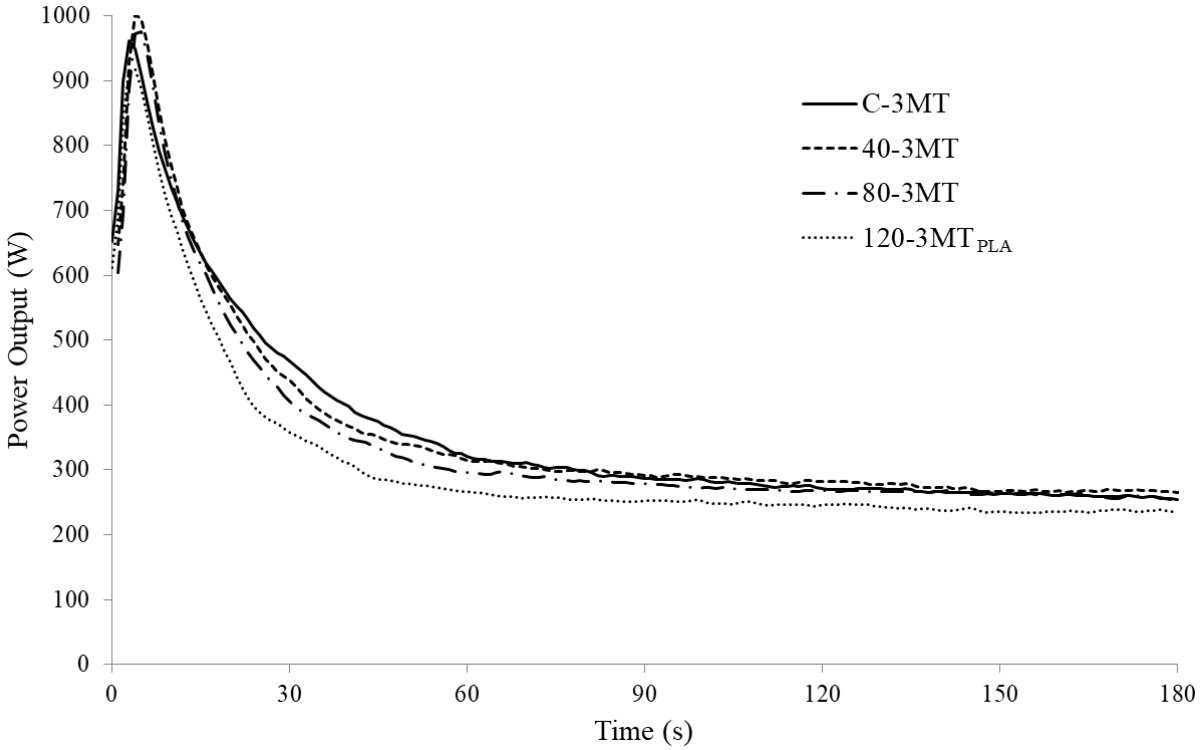



***Figure 1.*** *The group mean power profiles during the 3-min all-out test (3MT) measured with no prior exercise (C-3MT), and when preceded by 40 min (40-3MT), 80 min (80-3MT) and 2 h (120-3MT$_{PLA}$) of heavy-intensity exercise. Error bars are not shown for clarity. The end test power was significantly reduced at 2 h compared to control and the work done above the end test power was significantly reduced at 80 min and at 2 h. See text for further details.*

The power output profiles during the C-3MT, 40-3MT, 80-3MT and 120-3MT$_{PLA}$ are shown in Fig. 1. There were no differences in $\dot{V}O_{2peak}$ between C-3MT (4.03 ± 0.40 L·min$^{-1}$), 40-3MT (4.19 ± 0.40 L·min$^{-1}$), 80-3MT (4.15 ± 0.41 L·min$^{-1}$), 120-3MT$_{PLA}$ (4.08 ± 0.51 L·min$^{-1}$) and the ramp incremental test ($P$>0.05). There was no differences in EP between C-EP (260 ± 37 W), 40-EP (268 ± 39 W) and 80-EP (260 ± 40 W; $P$>0.05). However, 120$_{PLA}$-EP was lower than EP in all the other conditions (236 ± 47 W; $P$<0.05; Fig. 2A). There was no difference in WEP between C-WEP (17.9 ± 3.3 kJ) and 40-WEP

(16.1 ± 3.3 kJ), but both 80-WEP (14.7 ± 2.9 kJ; $P$<0.05) and $120_{PLA}$-WEP (13.8 ± 2.7 kJ; $P$<0.05) were lower than C-WEP; 80-WEP was also lower than 40-WEP ($P$<0.05; Fig. 2B). Results for peak power output and TWD during the 3MTs are shown in Fig. 2C and Fig. 2D, respectively. Blood [lactate] immediately after the 3MT was higher in the 40-3MT (8.8 ± 2.1 mM) compared to the 120-$3MT_{PLA}$ visit (7.0 ± 2.1 mM; $P$<0.05) with no difference after any other 3MTs.

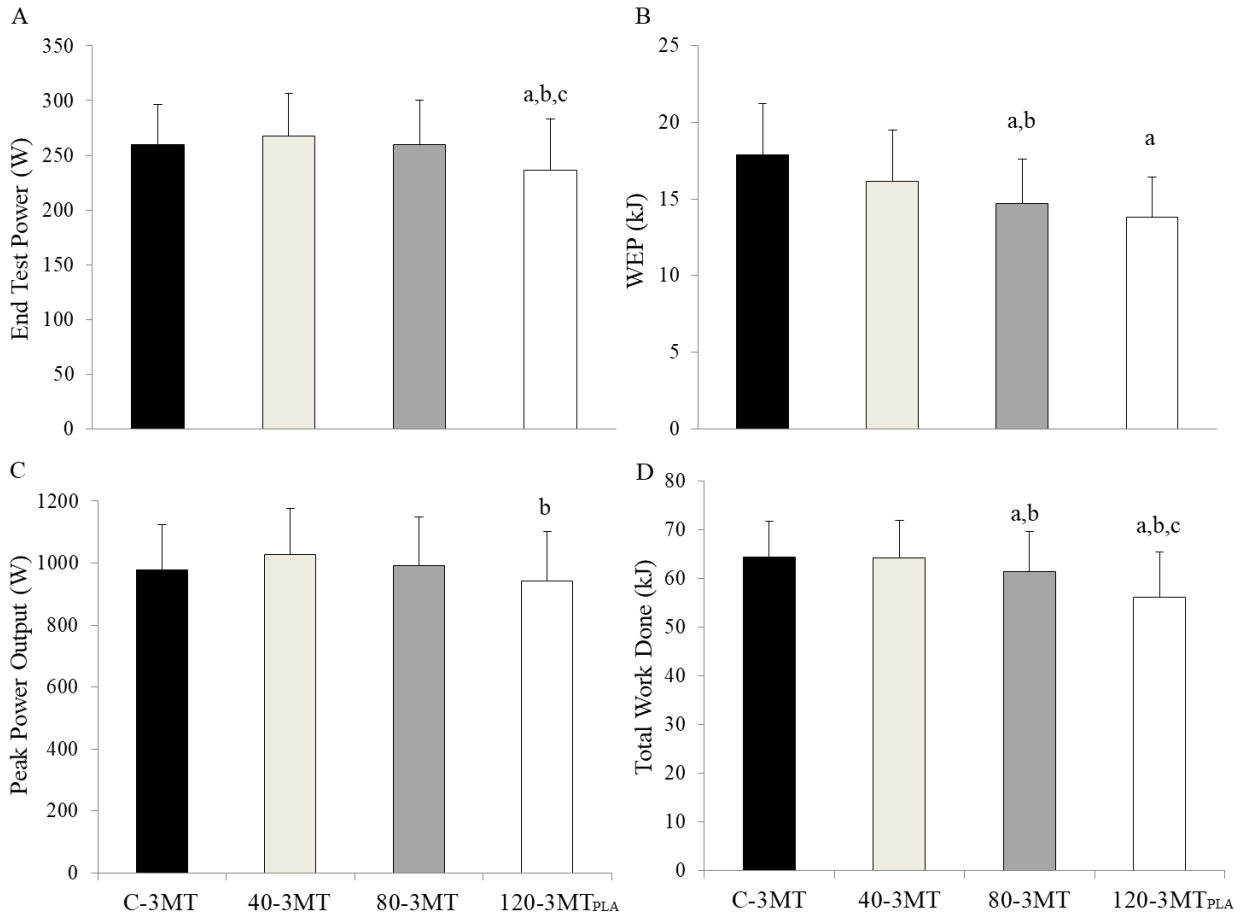



***Figure 2.*** *Group mean end test power (A), work done above end test power (B), peak power outputs (C) and total work done (D) during the 3-min all-out test (3MT) measured*

*with no prior exercise (C-3MT), and following 40 min (40-3MT), 80 min (80-3MT) and 2 h (120-3MT$_{PLA}$) of heavy-intensity exercise. a = different from C-3MT (P<0.05), b = different from 40-3MT (P<0.005), c = different from 80-3MT (P<0.05).*

*Experiment II: Influence of CHO ingestion on changes in the power-duration relationship following 2 h of heavy-intensity exercise*

In both the 120-3MT$_{PLA}$ and 120-3MT$_{CHO}$ tests, the relative intensity increased from ~64% $\dot{V}O_{2peak}$ at 10-15 min to ~68% $\dot{V}O_{2peak}$ at 115-120 min (Fig. 3A). HR increased during both 120-3MT visits (Table 1). There was no difference in relative intensity or HR between the 120-3MT$_{CHO}$ and 120-3MT$_{PLA}$ visits at any time point (Table 1 and Fig. 3A). RER decreased during both the 120-3MT$_{CHO}$ and 120-3MT$_{PLA}$ visits, but was higher at 115-120 min in the 120-3MT$_{CHO}$ trial compared to the 120-3MT$_{PLA}$ (Fig. 3B). CHO oxidation was higher in the 120-3MT$_{CHO}$ bout compared to the 120-3MT$_{PLA}$ bout at 85-90 and 115-120 min (Fig. 3C). Blood [glucose] was higher during the 120-3MT$_{CHO}$ bout compared to 120-3MT$_{PLA}$ at all time points at and beyond 40 min (Fig. 3D). There was no difference in blood [lactate] measured during the 120-3MT$_{CHO}$ and 120-3MT$_{PLA}$ trials. However, blood [lactate] was higher post 3MT during the 120-3MT$_{CHO}$ (8.4 ± 0.5 mM) compared to the 120-3MT$_{PLA}$ visit (7.2 ± 0.5 mM; $P$<0.05).

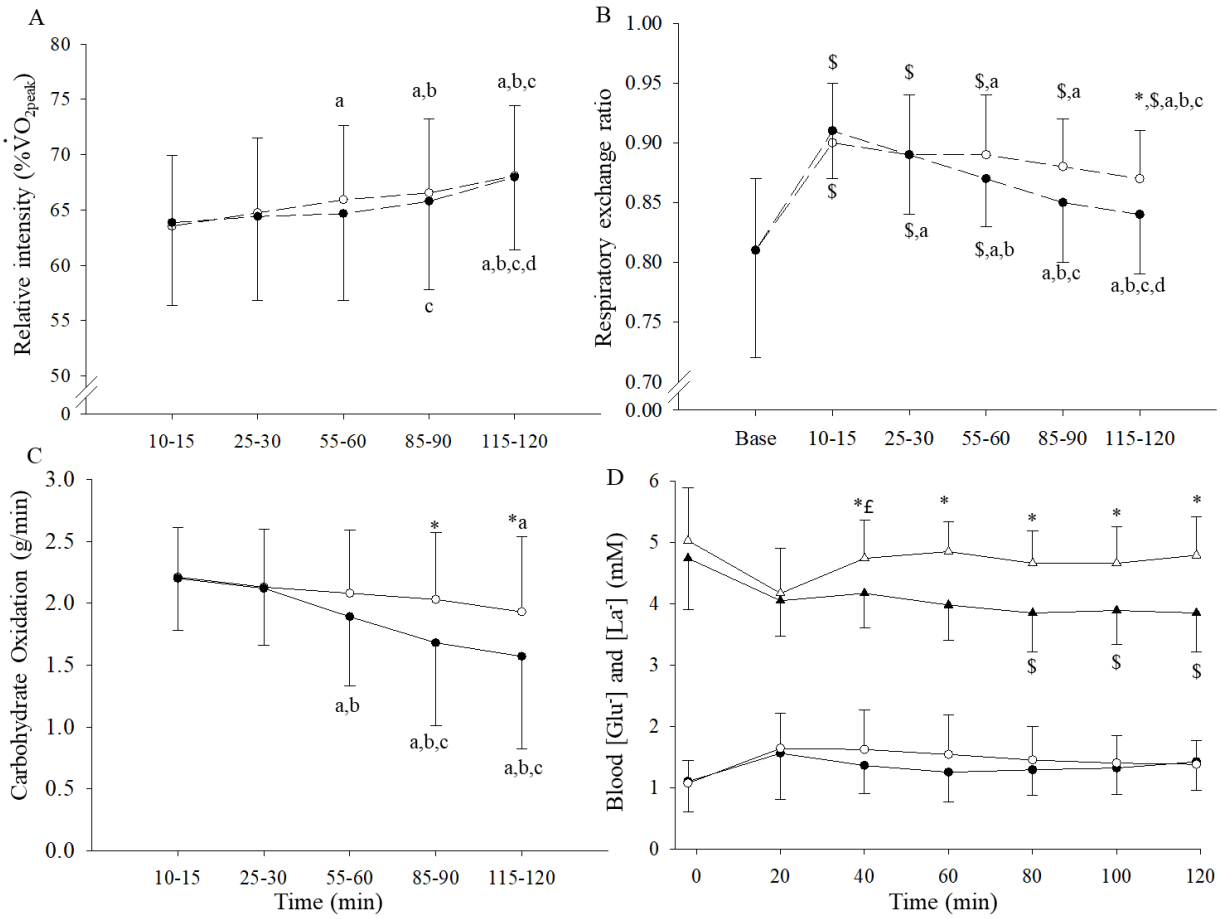



***Figure 3.*** *Group mean relative intensity (%$\dot{V}O_{2peak}$) (panel A), respiratory exchange ratio (panel B), carbohydrate oxidation (panel C) and blood [glucose] and [lactate] (panel D) during 2 h of heavy-intensity cycling while ingesting carbohydrate (white symbols) or placebo (black symbols). * = different from placebo (P<0.05), a = different from 10-15 min (P<0.05), b = different from 25-30 min (P<0.05), c = different from 55-60 min (P<0.05), d = different from 85-90 min (P<0.05), $= different from baseline (P<0.05), £ = different from 20 min (P<0.05).*

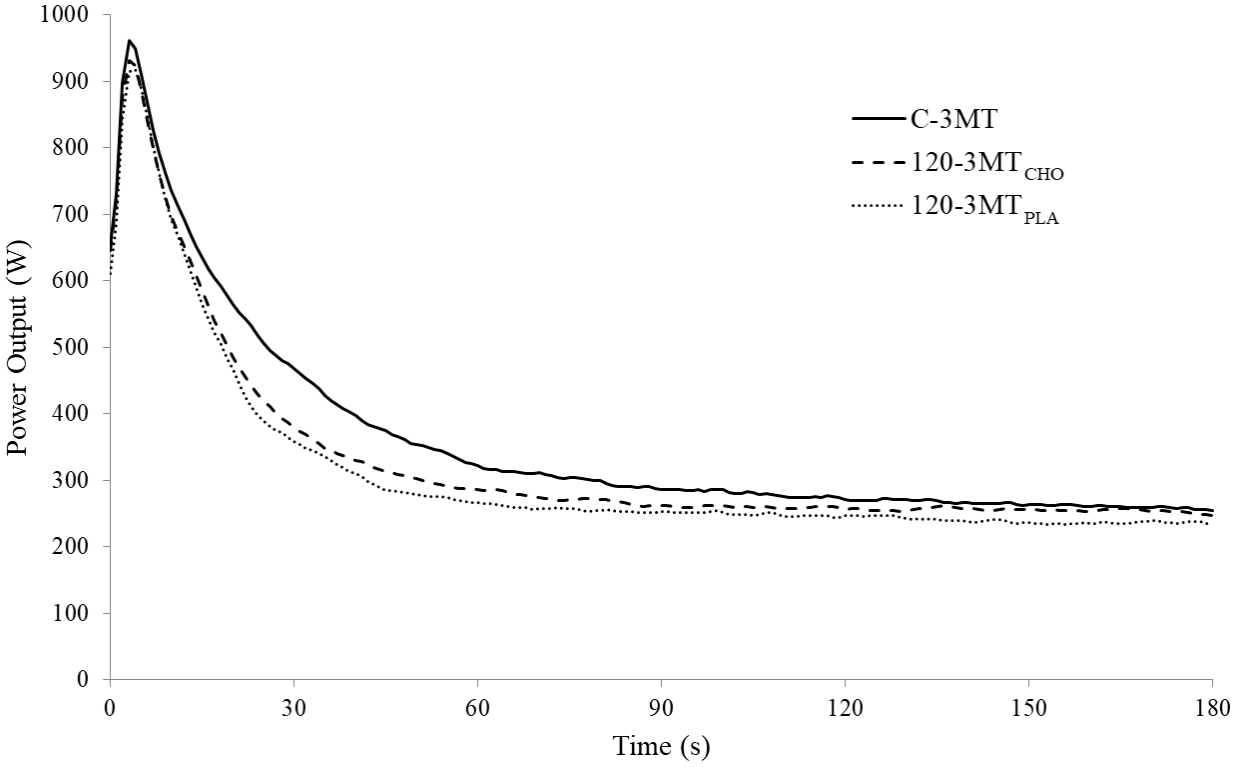
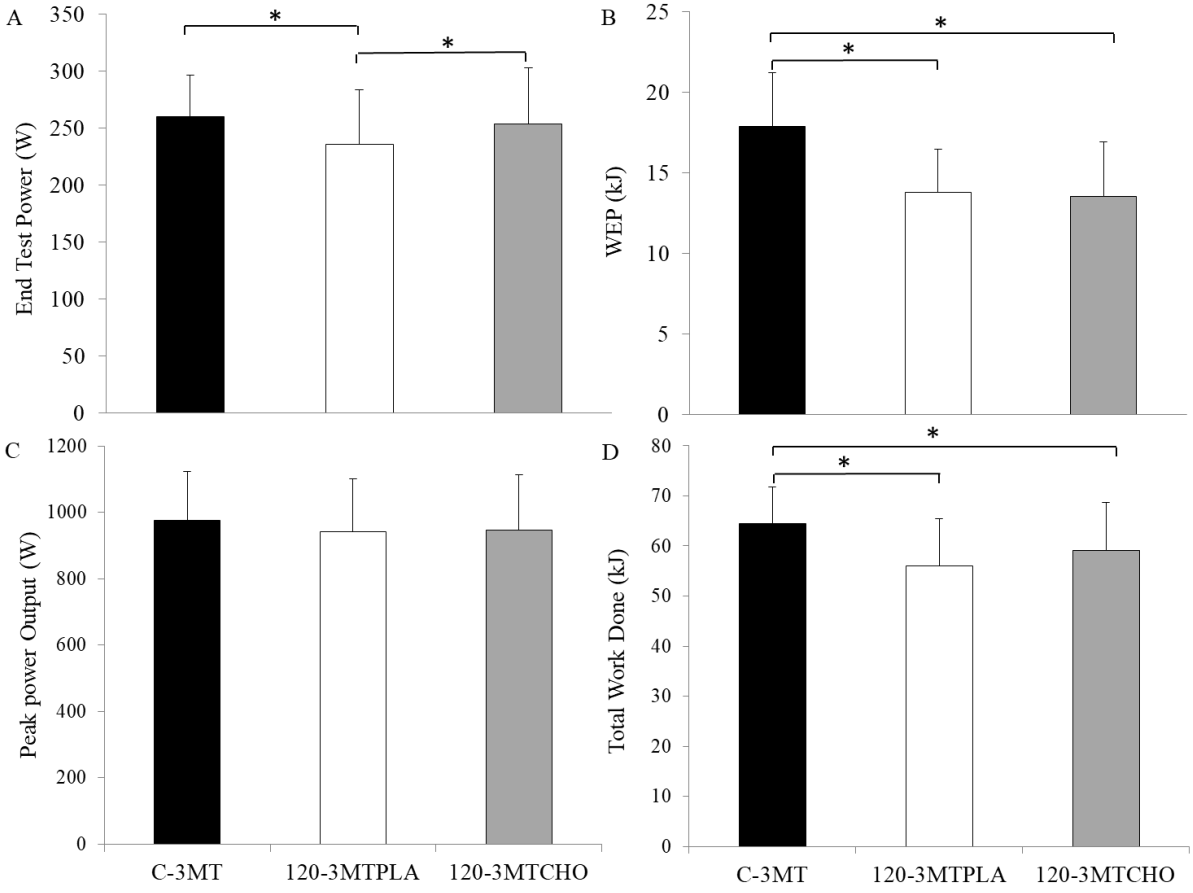



***Figure 4.*** *The group mean power profiles during the 3-min all-out test (3MT) measured with no prior exercise (C-3MT) and when preceded by a 2 h-heavy intensity exercise bout with ingestion of carbohydrate (120-3MT$_{CHO}$) or placebo (120-3MT$_{PLA}$). Error bars are not shown for clarity. The end test power was significantly reduced at 2 h compared to control when placebo was ingested but was not different to control when carbohydrate was ingested. The work done above the end test power was significantly reduced compared to control irrespective of placebo or carbohydrate ingestion. See text for further details.*

The power output profiles during the C-3MT, 120-3MT$_{CHO}$ and 120-3MT$_{PLA}$ are shown in Fig. 4. There were no differences in $\dot{V}O_{2peak}$ between C-3MT, 120-3MT$_{CHO}$, 120-3MT$_{PLA}$ and the ramp incremental test ($P$>0.05). 120$_{PLA}$-EP was 9% lower (236 ± 47 W) than C-EP (260 ± 37 W; $P$<0.05) and 7% lower than 120$_{CHO}$-EP (254 ± 49 W; $P$<0.05; Fig. 5A).

$120_{CHO}$-EP was not different from C-EP (Fig. 5A). C-WEP was 22% higher (17.9 ± 3.3 kJ) than $120_{PLA}$-WEP (13.8 ± 2.7 kJ; $P$<0.001) and 24% higher than $120_{CHO}$-WEP (13.5 ± 3.4 kJ; $P$<0.001; Fig. 5B). $120_{CHO}$-WEP was not different from $120_{PLA}$-WEP (Fig. 5B).




Results for peak power output and TWD are shown in Fig. 5C and Fig. 5D, respectively.

***Figure 5.*** *Group mean ± SD end test power (A), work done above end test power (B), peak power outputs (C), and total work done (D) during the 3-min all-out test measured with no prior exercise (C-3MT) and after 2 h of exercise while ingesting carbohydrates (120-3MT$_{CHO}$) or placebo (120-3MT$_{PLA}$). * = significant difference (P<0.05).*

There was insufficient muscle tissue in one biopsy sample for completion of muscle glycogen analyses, and therefore the muscle glycogen data are for n=14. Muscle [glycogen] decreased over the 2 h heavy-intensity exercise bouts in both the 120-3MT$_{CHO}$ and 120-3MT$_{PLA}$ trial (Fig. 6). Muscle [glycogen] was lower following the 2 h bout in the 120-3MT$_{PLA}$ trial compared to 120-3MT$_{CHO}$ trial; however, the change in muscle [glycogen] was not different between trials (Fig. 6). There was no correlation between the change in muscle [glycogen] over the 2 h exercise bout and the changes in EP or WEP in either the 120-3MT$_{PLA}$ or the 120-3MT$_{CHO}$ bout.

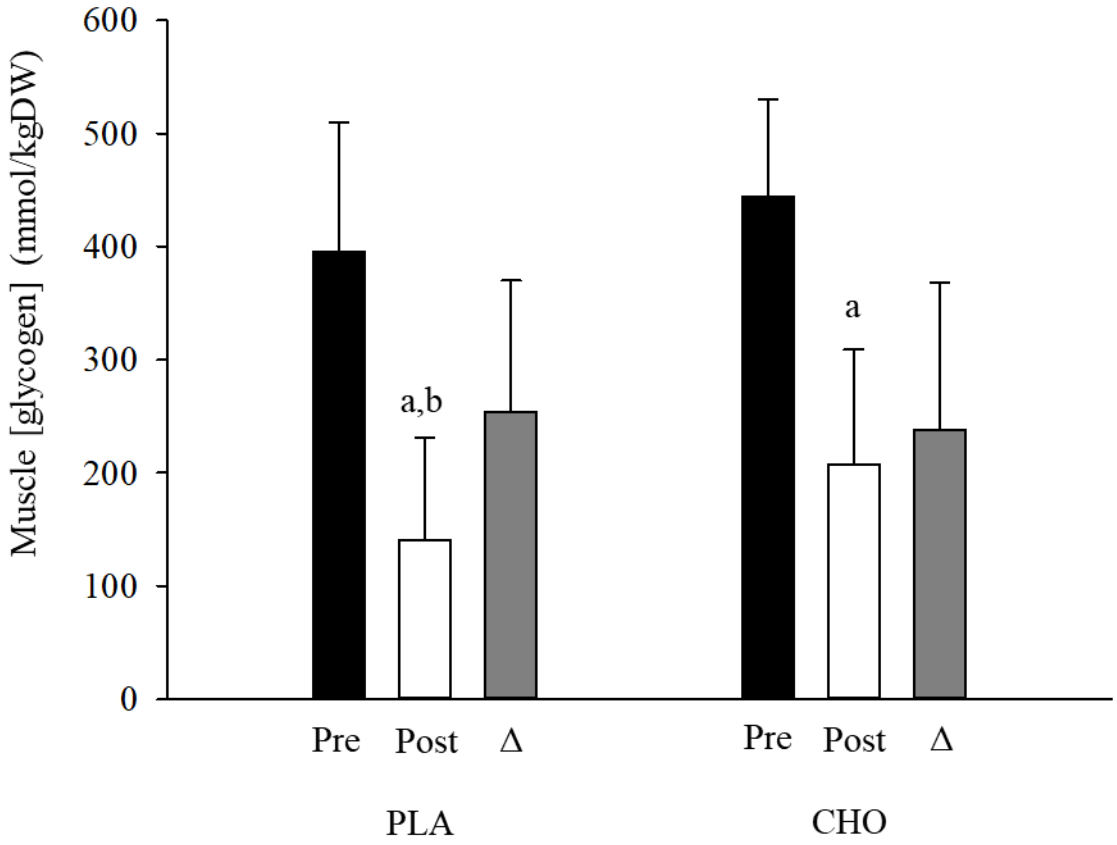


***Figure 6.*** *Group mean ± SD muscle [glycogen] before and after 2 h of heavy-intensity exercise while consuming carbohydrate (CHO) or placebo (PLA). a = different from pre-exercise values (P<0.001), b = different from CHO condition (P<0.05).*

To increase sample size and therefore increase confidence in our analysis, we combined the data from the present study with those from Clark et al. (13) to create a data set containing 28 participants who underwent muscle biopsies and completed a 3MT both at rest and following 2 h of heavy-intensity exercise. The pooled data show that muscle [glycogen] at rest was correlated with control EP (Fig. 7A) and that muscle [glycogen] following 2 h of heavy-intensity exercise was correlated with both fatigued EP (Fig. 7B) and fatigued WEP ($r$ = 0.43; $P$<0.05). The change in EP following 2 h of heavy-intensity exercise was not correlated with the change in muscle [glycogen] (Fig. 8C, 8D). However, the percentage change in muscle [glycogen] following 2 h of heavy-intensity exercise was correlated with both the absolute change in WEP (Fig. 7E) and the percentage change in WEP (Fig. 7F).

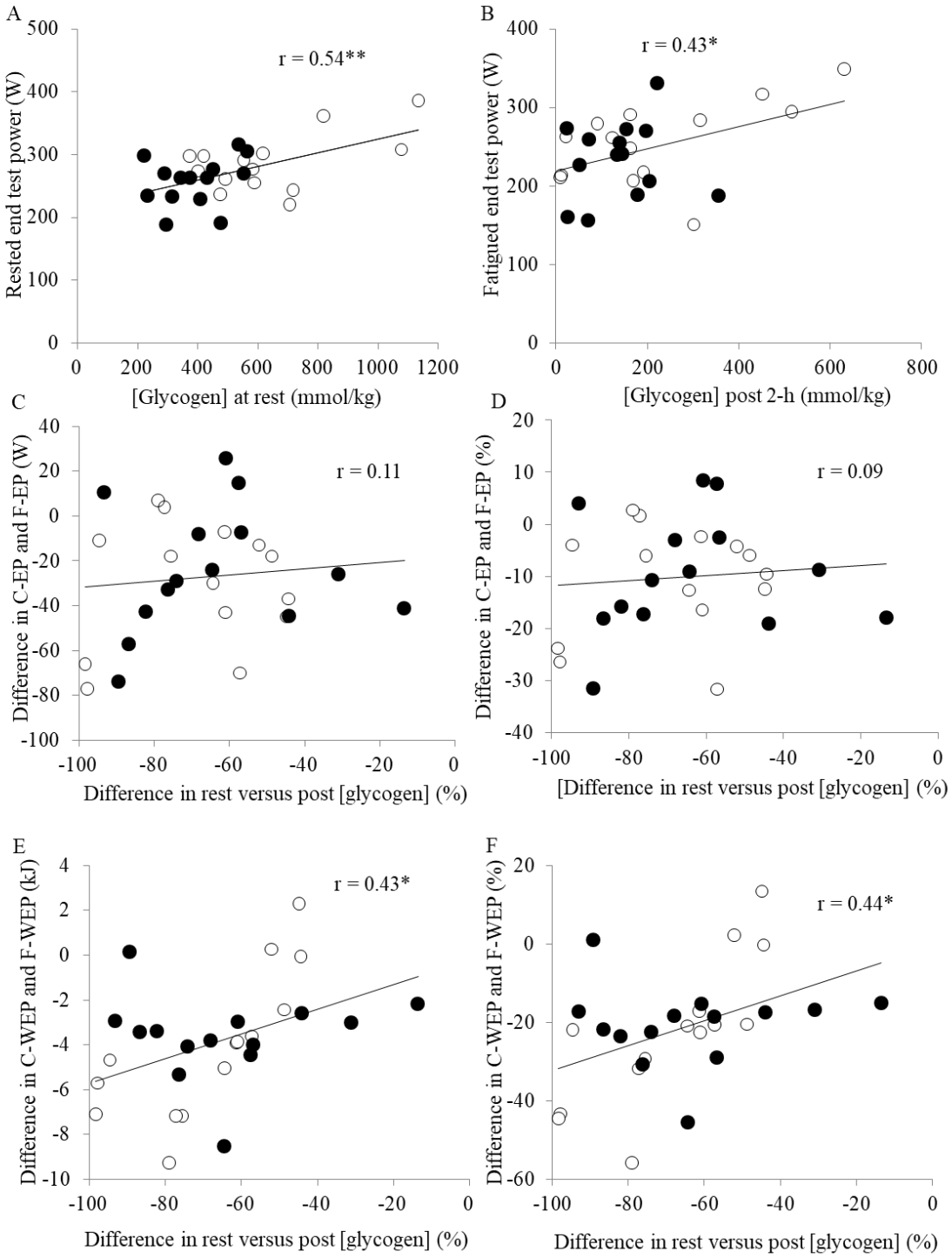



***Figure 7.*** *Combined results from present study (black circles) and Clark et al. (13) (white circles) (n=28). Correlation between muscle [glycogen] at rest and end test power (EP) without preceding exercise (panel A) and correlation between muscle [glycogen]*

*and EP after 2 h of heavy-intensity exercise (B). Correlations between the percentage change in muscle [glycogen] and the absolute (panel C) and percentage (panel D) change in EP over 2 h of heavy-intensity exercise. Correlations between the percentage change in muscle [glycogen] and the absolute (panel E) and percentage (panel F) change in WEP over 2 h of heavy-intensity exercise. ** = P<0.005; * = P<0.05*

## 7.4 Discussion

This is the first study to investigate the influence of different durations of prolonged endurance exercise, and CHO ingestion during exercise, on the parameters of the power-duration relationship. We used the 3MT as a practical and expeditious method to appraise changes in the power-duration relationship because it has been shown that the EP and WEP provide valid estimates of CP and W′, respectively, both in the absence of prior exercise (7, 48) and following 2 h of heavy-intensity cycle exercise (13). The principal novel findings were that: (1) EP was not altered by 40 min or 80 min but was significantly reduced after 2 h of heavy-intensity exercise; (2) WEP was not altered by 40 min but was significantly reduced after 80 min and 2 h of heavy-intensity exercise; (3) the reduction in EP following 2 h of heavy-intensity exercise was negated when CHO was consumed; and (4) the reduction in WEP following 2 h of heavy-intensity exercise was not rescued by CHO ingestion. These results reveal disparate time courses for the changes in EP and WEP during heavy-intensity exercise: WEP appears to fall in an approximately linear fashion and becomes significantly reduced when exercise duration ≥80 min; while EP is preserved for at least 80 min and is only significantly reduced

when exercise duration approaches 2 h. Importantly, our results show that EP (but not WEP) is preserved following 2 h of heavy-intensity exercise when 60 g/h CHO is consumed, suggesting that CP (but not W′) is influenced, at least in part, by CHO feeding. These findings may have significant implications for performance prediction and the formulation of optimal race (pacing and nutritional) strategies.

*I: Dynamic changes in the parameters of the power-duration relationship following 40 min, 80 min and 2 h of heavy-intensity exercise*

Consistent with our previous research, we found that 2 h of heavy-intensity exercise led to a decline of both EP and WEP (12, 13). Specifically, there was a ~9% fall in EP and a ~22% fall in WEP compared to C-3MT, results which are in close agreement with our previous findings of an 8-11% reduction in EP and a 20-22% reduction in WEP following 2 h of heavy-intensity exercise (12, 13). The key novel findings of the present study were that there were no significant changes in either EP or WEP compared to the control condition following 40 min of heavy-intensity exercise whereas, following 80 min of exercise, EP was unchanged but WEP was significantly reduced (by 17%). Our study therefore reveals differences in the time course of changes in EP and WEP during endurance exercise, with WEP deteriorating more rapidly than EP. These results may provide insight into the physiological mechanisms underpinning the power-duration relationship and their relationship with the fatigue process during endurance exercise.

Several factors likely contribute to the changes observed in the power-duration parameters following 80 min and/or 2 h of heavy-intensity exercise. An increase in the $O_2$ cost of sustaining the constant power output was observed during the 2 h (from

~64% to ~68% of initial $\dot{V}O_{2peak}$) and 80 min (from ~63% to ~65% of initial $\dot{V}O_{2peak}$) but not the 40 min exercise bout (stable at ~63% of initial $\dot{V}O_{2peak}$) and occurred concomitantly with a decrease in RER in the 2 h (from ~0.91 to ~0.84) and 80-min (from ~0.93 to ~0.91) exercise bouts but not in the 40 min bout (from ~0.93 to ~0.92). The progressive loss of efficiency is therefore related in part to a shift in substrate utilisation from CHO towards fat oxidation during the 80 min and 2 h exercise bouts. Given that EP reflects a critical oxidative metabolic rate (1), a loss of efficiency (i.e., a higher $\dot{V}O_2$ per watt of power output) would necessarily result in a reduced EP.

A substantially increased core temperature and consequent redistribution of blood flow away from skeletal muscle to facilitate heat exchange might also increase the overall $O_2$ cost of exercise (36). Moreover, significant dehydration, due to the sweat rate exceeding the rate of fluid replacement, could compromise cardiac output and muscle $O_2$ delivery (42). Given that EP is an index of oxidative metabolic function (29, 47), these changes could reduce efficiency and/or exacerbate muscle metabolic perturbation and fatigue development. In our study, however, participants exercised in an air-conditioned lab ($20^{o}$ C) and consumed 380 ml of fluid per hour, *pro rata*, such that body mass losses were minimal (≤1.0 kg; ≤1.2%). Such small changes in body mass suggest that issues related to thermoregulation probably did not contribute appreciably to the decline in 3MT performance we observed and certainly indicate that, if hyperthermia did occur, it likely affected subjects similarly.

It appears that CP represents an important boundary for neuromuscular fatigue development (9, 40) such that central fatigue, which may be determined by a reduction

in voluntary activation measured by motor nerve stimulation, makes a greater contribution to fatigue development in the heavy-intensity domain compared to the severe-intensity domain (4, 9, 46). It is possible, therefore, that the development of central fatigue during the 80 min and 2 h heavy-intensity exercise bouts in the present study impaired performance in the subsequent 3MT, reducing EP and/or WEP. Consistent with this interpretation, it has been reported that acetaminophen ingestion, which would be expected to blunt central fatigue development by attenuating the sensation of pain and preventing any reduction in central motor drive, led to greater muscle activation over the last 30 s of a 3MT and a higher EP compared to the placebo condition (31).

The reduction in WEP after just 80 min as well as after 2 h of heavy-intensity exercise is intriguing. Significant changes in muscle [ATP] and/or muscle metabolic status (i.e. substantial depletion of phosphocreatine or accumulation of hydrogen ions) would not be anticipated during heavy-intensity exercise (4) such that the development of peripheral fatigue through these mechanisms is unlikely to explain the reduced WEP. The lack of effect on peak power output during the 3MT following 80 min and 2 h of heavy-intensity exercise might suggest that the reduced WEP at these time points is related not to high-energy phosphate depletion but rather to impaired ATP production via anaerobic glycolysis which may, in turn, be related to muscle glycogen depletion. Consistent with this, a reduction in WEP has been reported when glycogen depletion has been invoked by dietary restriction (30) or the performance of 2 h of heavy-intensity exercise (13). We note here that the evaluation of WEP is less reliable than EP (12) such that possible error in the estimates of WEP is a possible limitation of the present

study; however, the reduction in WEP at 80 min and 2 h of exercise is substantial relative to the likely error.

When we combined the data from the present study with those from Clark et al. (13), we found a significant correlation between the change in WEP, but not EP, and the change in muscle [glycogen] following 2 h of heavy-intensity exercise. The progressive decline in muscle [glycogen] that would be expected during prolonged heavy-intensity exercise (18, 19) could therefore contribute to the reduction in WEP during the 80 min and 2 h exercise bouts. It is interesting to note, however, that WEP was significantly reduced despite muscle glycogen depletion being far from complete (~36% of the resting value was remaining following 2 h of exercise) at the start of the 3MT. An important consideration in this regard is that muscle glycogen depletion can be localised to subcellular compartments such that muscle performance might become impaired even when 'global' muscle [glycogen] appears sufficient (34). The present data suggest that absolute muscle [glycogen] may influence the rate of energy generation through anaerobic glycolysis, and/or influence contractile function via effects on sarcoplasmic reticulum calcium release (37), thereby reducing WEP.

*II: Influence of CHO ingestion on changes in the power-duration relationship following 2 h of heavy-intensity exercise*

We tested the hypothesis that changes in the power-duration relationship following 2 h of heavy-intensity exercise would be mitigated when CHO, compared to placebo, was consumed during exercise. A striking observation was that the reduction in EP following 2 h of heavy-intensity exercise was eliminated by 60 g/h of CHO ingestion. Specifically,

in the placebo condition, EP was reduced by 9% compared to C-EP, consistent with previous findings (12, 13); however, when CHO was ingested during exercise, there was no significant reduction in EP compared to C-EP. In contrast, WEP was significantly reduced in both the placebo and CHO conditions (22% and 24% reductions, respectively) compared to C-WEP, with there being no difference between the two conditions. An important novel finding of the present study is therefore that EP, but not WEP, may be preserved by CHO ingestion during endurance exercise of up to 2 h duration.

Significant muscle glycogen depletion was evident following 2 h of heavy-intensity exercise both when subjects ingested CHO (53% reduction in [glycogen]) and when they ingested a placebo beverage (64% reduction in [glycogen]). Muscle [glycogen] was higher following 2 h of heavy-intensity exercise with CHO compared to placebo ingestion but this may be explained by the tendency for muscle [glycogen] to be higher at baseline in the CHO condition. The reduction in muscle [glycogen] over the 2 h exercise bout was not different between conditions despite there being greater CHO availability and utilization (evidenced by higher RER, a greater rate of CHO oxidation and higher blood [glucose]) when CHO was ingested. Similarly, Smith et al. (45) reported that ingestion of 60 g/h of CHO during 2 h of exercise at 77% of $\dot{V}O_{2peak}$ did not spare muscle glycogen, compared to placebo, but did enhance subsequent 20-km time trial performance, an effect that the authors suggested was due to the increased rate of CHO oxidation. In light of the results of the present study, it is possible that the

participants in Smith et al. (45) had a higher CP and could therefore sustain a higher power output during the 20-km time trial following CHO compared to placebo ingestion.

The increased rate of CHO oxidation observed in the present study, consequent to CHO ingestion, likely contributed to the preserved EP we observed. However, given the greater proportional development of central fatigue during heavy-intensity endurance exercise (4, 9, 46), it is possible that other factors also contributed. In the placebo condition, blood [glucose] was significantly lower than the baseline value at 100 and 120 min of exercise (~3.8 mM) whereas it remained stable throughout exercise in the CHO condition (~4.8 mM). It is possible that this difference in blood [glucose] attenuated central fatigue development (14, 35) and enabled a higher muscle activation during the subsequent 3MT. Similarly, the detection of CHO by sensors in the mouth, even when the CHO is not swallowed, may attenuate decrements in exercise performance associated with fatigue (10, 16, 22). Specifically, it appears that CHO receptors in the oral cavity may signal an impending increase in CHO availability to higher brain regions (11, 16, 23). It is possible therefore that, in addition to direct effects on skeletal muscle metabolism, the ingestion of CHO during exercise reduced central fatigue and enabled enhanced motor output and contractile function (35) during the subsequent 3MT.

In the present study, no significant correlations were found between the changes in muscle [glycogen] and EP or WEP in either condition, possibly due to the relatively small sample size. However, when the data from the present study were pooled with those of Clark et al. (13), we found a positive relationship between EP and muscle [glycogen] both at rest and following 2 h of heavy-intensity exercise. These results

suggest a link between aerobic fitness and muscle glycogen storage, which might be mediated by training status (15). Moreover, when the data from the two studies were combined, we found that, following 2 h of heavy-intensity exercise, muscle [glycogen] was correlated with WEP and also that the change in muscle [glycogen] over the 2 h exercise bout was correlated with the change in WEP.

The physiological mechanisms underpinning W′ have not been entirely resolved (5, 33, 43). However, because CP is closely related to the proportion of type I muscle fibers (29, 47), it has been suggested that W′ may reflect the metabolic, contractile and/or fatigue-related characteristics of type II muscle fibers (47, 51). The fall in WEP we observed might therefore reflect the specific effects of 2 h of heavy-intensity exercise on the type II fiber population, including glycogen depletion (52). There is evidence to suggest that W′ is related to the capacity for substrate-level phosphorylation, with PCr availability being important for the achievement of peak power output in the 3MT (39, 47, 49). It has been reported that W′ is reduced following glycogen depletion induced by dietary CHO restriction, with there being no effect on CP (30). The results of the current investigation are consistent with these findings in showing that WEP is reduced when glycogen depletion is evident following 2 h of heavy-intensity cycling, irrespective of effects on EP.

The different effect of CHO feeding during endurance exercise on EP and WEP provide novel insight into the mechanisms underpinning these parameters (and, by extension, CP and W′). While muscle glycogen depletion was not altered compared to the placebo condition, the maintenance of euglycaemia by CHO ingestion during prolonged exercise

resulted in a preservation of EP in the face of a decline in WEP. This suggests that, following 2 h of heavy-intensity exercise, EP may be modulated by central fatigue whereas WEP may be more directly related to muscle glycogen availability. Further studies are required, with more direct measurements of central and peripheral fatigue, to explore this possibility.

*Implications for Performance*

The results of the present study have several potentially important implications for athlete performance diagnostics and race practice. The disparate time courses for the degradation of EP and WEP we observed during prolonged endurance exercise is of particular interest. The maintenance of EP for ~80 min of such exercise indicates that performance for events which are sustained below EP can be faithfully predicted from measurements made in the rested state whereas, for longer events, the fall in EP render such predictions more complicated. Indeed, inter-individual variation in the reductions in EP and WEP *during* endurance exercise, reflecting fatigue resistance, is likely an important but previously overlooked component of success in endurance sports (12). The fall in WEP during heavy-intensity exercise occurred much earlier than the fall in EP, with the group mean reduction being 10% after 40 min (not significant), 18% after 80 min and 23% after 2 h. This relatively early reduction in WEP may impact on an athlete's ability to draw upon W′, limiting their ability to respond to, or initiate, surges in pace above CP, a factor that should be carefully considered when developing race tactics.

Our findings also emphasise the importance of CHO consumption during prolonged endurance exercise by showing that ingestion of 60 g/h CHO enabled EP to be maintained compared to a 9% reduction in EP when placebo was consumed. The performance implications of this difference, whether measured in terms of the mean speed that may be sustained throughout a race or in the ability to finish strongly, are likely to be profound. Depending on the power being sustained and the extent of the fall in CP during prolonged endurance exercise, it is possible that an athlete may eventually transition from the heavy- to the severe-intensity exercise domain, with the inevitable outcome that power must fall and/or exercise will become intolerable. This highlights the important interaction between physiology and nutrition during endurance exercise. Maintaining a high rate of CHO ingestion while exercising at a high intensity is an important consideration, and a potential limiting factor, for extreme human endurance challenges such as the attempt to run a <2 h marathon (6, 27).

It is important to acknowledge that our results are specific to the conditions of our study. Naturally, dynamic changes in EP and WEP would likely be different if other combinations of exercise intensity and duration were assessed. Moreover, the effect of CHO ingestion on the change in EP is also likely influenced by exercise intensity, exercise duration and the rate (and type) of CHO consumption (22). Finally, the extent to which our results in cycling may be extrapolated to other exercise modalities is presently unclear. These questions and the nature of the interaction between these key variables might be the subject of future investigations.

*Conclusion*

In conclusion, the EP and WEP, surrogates of the parameters of the power-duration relationship, CP and W', respectively, were reduced following 2 h of heavy-intensity cycling. WEP, but not EP, was also reduced following 80 min of heavy-intensity cycling. CHO ingestion during 2 h of heavy-intensity cycling abolished the reduction in EP, but not WEP. These results suggest that, following prolonged endurance exercise, W' may be sensitive to local muscle glycogen availability, presumably via a limitation to energy production by anaerobic glycolysis, whereas CP may be sensitive to global CHO availability, perhaps via its relationship with central fatigue development. Practitioners should be aware that dynamic changes in the parameters of the power-duration relationship *during* heavy-intensity exercise present a challenge to the use of these parameters to predict performance during endurance sports events. It is clear, however, that CHO supplementation represents a practical and effective intervention to constrain the deterioration of CP during endurance exercise.

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# Chapter 8. General Discussion

A comprehensive understanding of fatigue, including psychobiological and physiological factors can inform successful interventions to offset fatigue and improve exercise performance in a number of exercise settings and athletic populations. The majority of studies investigating how these factors modulate exercise performance have been conducted using recreationally active individuals resulting in limited application to highly trained athletes. This thesis compared the regulation of exercise tolerance by psychobiological factors between untrained individuals and competitive athletes. Furthermore, the parameters of the power-duration relationship, CP and W′, are regularly used as a tool to understand the physiological responses to exercise of different intensities and to predict performance in a given event. Often, this process consists of using the CP and W′ parameters estimated from short bouts of exercise in a rested state and applying them to predict how that same individual will perform following periods of long-duration, fatiguing activity. However, it is possible that the CP and W′ are affected by the processes of fatigue that occur during long-duration events thus limiting the accuracy of performance prediction. Therefore, this thesis investigated the influence of fatiguing endurance exercise on the parameters of the power-duration relationship.

## 8.1 Research questions addressed

The aim of this thesis was twofold. Firstly, to investigate the possible psychobiological factors contributing to fatigue in competitive athletes, and secondly, to investigate the

effect of prolonged fatiguing exercise on the power-duration parameters. The following questions were addressed:

1) How does a prolonged cognitive task affect subsequent exercise performance in untrained individuals and competitive athletes?
   - Is the exercise performance of untrained or competitive athletes affected by a prolonged cognitive function task?
2) What are the effects of 2 h of fatiguing heavy-intensity exercise on CP and W'?
   a. Is the 3MT a reliable test for estimating EP and WEP when administered in a fatigued state?
   b. Does EP estimated following 2 h of heavy-intensity exercise demarcate the heavy and the severe exercise domains?
   c. Do the CP and W' estimates derived from the 3MT and the conventional prediction trial protocols differ after 2 h of heavy intensity exercise?
3) What is the time course over which EP and WEP deteriorate during prolonged endurance exercise?
4) Does carbohydrate feeding eliminate possible changes in CP and W' after 2 h of heavy-intensity exercise?

## 8.2 Summary of main findings

### 8.2.1 Prolonged cognitive task does not alter exercise performance in competitive athletes

The novel finding from chapter 4 was that a 6 min TT performance was not affected by a 30 min cognitive functioning task in either competitive athletes or in untrained

individuals. Additionally, chapter 4 found that there were no differences in the physiological measurements taken ($\dot{V}O_2$, heart rate or blood [lactate] responses) during the 6 min bout after completing a cognitive function task. This suggests that constant work-rate and maximal self-paced exercise that is performed in the severe-intensity domain are not affected by a prolonged cognitive functioning task irrespective of training status.

The purpose for the next study of the thesis was to investigate the role psychological stress and physiological fatigue in form of endurance exercise has on the power-duration relationship. Due to the lack of effect on severe-intensity exercise performance following a cognitive function task it seemed reasonable not to pursue psychological stress as an intervention in subsequent studies in the present thesis. Therefore, the following chapters are investigating the effect endurance exercise has on the power-duration relationship.

### 8.2.2 The 3-min all-out exercise test is a reliable and valid test to estimate the parameters of the power-duration relationship in a fatigued state

Among the novel findings from chapters 5 and 6 were that the 3MT is a reliable test for determining a fatigued EP (F-EP) and WEP (F-WEP) after 2 h of heavy-intensity exercise and that these parameters are not significantly different from estimates derived using the conventional protocol. Chapter 5 sought to examine the reliability of the 3MT after prolonged fatiguing exercise and found that both F-EP and F-WEP were highly correlated ($r$ = 0.99) and did not differ from day to day. The coefficient of variation was 2% for F-EP and 6% for F-WEP. In chapter 6, 2 h of heavy-intensity exercise was

immediately followed by either a 3MT or 3 different time to exhaustion bouts to estimate F-EP and F-WEP or fatigued CP (F-CP) and W′ (F-W′), respectively. Chapter 6 demonstrated that there were no significant differences between the 3MT and the conventional protocol when estimating the parameters of the power-duration relationship in a fatigued state ($P$ > 0.05). F-EP showed a high accuracy of estimation for F-CP with an SEE of 17 W (7%), while, F-WEP and F-W′ had a limited agreement with an SEE of 4.4 kJ (29%) and therefore the fatigued 3MT (F-3MT) provides a more accurate estimate of F-CP compared to F-W′. These findings suggest that the 3MT can accurately estimate F-CP and subsequently evaluate change in CP after prolonged fatiguing exercise. WEP showed a greater test-retest variability than CP. However, it should be noted that W′ generally has a greater test-retest SEE compared to CP in a rested state also (Hill and Smith 1994).

### 8.2.3 The effects of prolonged fatiguing exercise on the power-duration relationship

Chapters 5, 6 and 7, for the first time, addressed how the parameters of the power-duration relationship are affected by 2 h of heavy-intensity exercise. Over chapters 5, 6 and 7 there were a total of 42 participants that performed a 3MT without prior exercise (control) and a 3MT preceded by 2 h of fatiguing exercise. These chapters showed a reduction in both EP and WEP after 2 h of heavy-intensity exercise compared to control. Pooled data from the 3 chapters showed that there was 9 % (~26 W) reduction in EP, compared to control ($P$ < 0.001; n=42; Table 8.1) and a 22% (~4.0 kJ) reduction in WEP compared to control after 2 h exercise ($P$ < 0.001; n=42; Table 8.1). Collectively,

chapters 5, 6, and 7 provide evidence that both parameters of the power-duration relationship decrease after 2 h of heavy-intensity exercise.

**Table 8.1.** *Combined chapter 5, 6 and 7 group mean end test power (A) and work done above end test power (B) during the 3-min all-out test in a rested and after 2 h of heavy-intensity exercise.*Different from rested condition (P < 0.001).*

| | EP | | | | WEP | | | |
|---|---|---|---|---|---|---|---|---|
| **Chapter** | Rested (W) | Fatigued (W) | Diff (W) | Diff (%) | Rested (kJ) | Fatigued (kJ) | Diff (kJ) | Diff (%) |
| 5 (study 2) | 306 | 282* | 24 | 8 | 18.3 | 14.7* | 3.5 | 20 |
| 6 (study 3) | 287 | 256* | 30 | 11 | 18.7 | 14.6* | 4.1 | 22 |
| 7 (study 4) | 260 | 236* | 24 | 9 | 17.9 | 13.8* | 4.1 | 22 |
| Mean | 282 | 256* | 26 | 9 | 18.3 | 14.3* | 3.9 | 22 |
| SD | 49 | 53 | 26 | 10 | 4.0 | 4.3 | 2.8 | 15 |

### 8.2.4 Time course of changes in the power-duration parameters during prolonged heavy-intensity exercise and influences of carbohydrate supplementation

Chapter 7, for the first time, investigated how 40 min and 80 min of heavy-intensity exercise affected the parameters of the power-duration relationship. Chapter 7 demonstrated that 40 min of heavy-intensity exercise is insufficient to alter EP and WEP. However, 80 mins was sufficient to reduce WEP by 17% but without affecting the EP. Furthermore, while both parameters were reduced at 2-h, WEP was reduced to a greater extent than EP (22% vs 9%). Together, these findings suggest that the WEP

may be more susceptible to change during long duration heavy-intensity exercise and deteriorate more rapidly than EP.

Additionally, in chapter 7, 60 g·min$^{-1}$ of carbohydrate was consumed during 2 h of heavy-intensity exercise to assess whether carbohydrate supplementation would mitigate the reduction seen in the parameters of the power-duration relationship after prolonged fatiguing exercise. Chapter 7 demonstrated that the reduction in EP measured after 2 h of fatiguing exercise was eliminated when a carbohydrate supplement was consumed. However carbohydrate supplementation did not attenuate the reduction in WEP (24%). These findings indicate that EP but not WEP may have a carbohydrate sensitivity during prolonged endurance exercise.

### 8.3 The physiological responses to exercise above and below end test power after 2 h of heavy-intensity exercise

Exercising below CP yields an attainment of a steady-state $\dot{V}O_2$ and blood [lactate] response, while in contrast, exercising above CP is associated with an increase in $\dot{V}O_2$, blood [lactate] and a limited exercise tolerance (Burnley and Jones 2007; Hill et al. 2002; Jones et al. 2010; Poole et al. 1988; Poole et al. 2016; Vanhatalo et al. 2016). A total of 23 participants in chapters 5 and 6 exercised, on separate occasions, both above and below F-EP or F-CP. All participants reached exhaustion before 30 min when exercising above F-EP and F-CP. In chapter 5, when exercising above F-CP, participants ceased exercise before the attainment of $\dot{V}O_{2peak}$ and with a blunted blood [lactate] response, while in chapter 6, participants reached $\dot{V}O_{2peak}$ values with higher blood [lactate] responses than those observed in the below F-CP trials. It should be

noted that the participants in chapter 6, on average, exercised at a higher work rate above F-CP compared to chapter 5. In chapter 5 participants exercised 15 W above F-EP (5 ± 1 % above F-EP), while in chapter 6, participants exercised (during their longest exercise bout within the severe-intensity domain) at work rates that were on average 25 ± 12 W above F-CP (10 ± 4 % above F-CP). It is presumed that the higher work rate in chapter 6 elicited a greater physiological response compared to chapter 5. When the participants exercised 15 W below F-EP (chapter 5) or F-CP (chapter 6) stable $\dot{V}O_2$ and blood [lactate] responses were detected, which is expected during exercise performed within the heavy-intensity domain. However, out of the 23 participants, only 8 completed a total of 30 min (a total of 16 participants completed >20 min) of exercise 15 W below F-EP or F-CP. In chapter 6, when muscle biopsy samples were collected before and after 2 h of heavy-intensity exercise, muscle [glycogen] was lower after 2-h in the participants reaching exhaustion before 20 min compared to the participants completing more than 20 min. However, owing to the small sample size there is insufficient data to perform statistical analysis in the form of correlation between time to exhaustion in the below F-CP bout and muscle [glycogen] post 2-h (2 participants reached exhaustion in <20min and 5 participants lasted 30 min and were therefore asked to stop exercising). Therefore, further research is required to investigate whether the amount of muscle [glycogen] after 2-h of heavy-intensity exercise reduces the ability to exercise below F-CP.

## 8.4 The influence of muscle glycogen on the power-duration parameters estimated in a 3-min all-out test

It is well established that muscle glycogen plays an imperative role as an energy substrate during long duration exercise and there is some evidence to suggest that the parameters of the power-duration relationship are influenced by muscle glycogen availability. In particular, Miura et al. (2000) investigated whether glycogen depletion, achieved via dietary restriction, would affect the parameters of the power-duration relationship. The authors found that W′ decreased by ~20% while CP remained unchanged following an evening before exercise trial (at 60% of $\dot{V}O_{2max}$ for 75 min, followed by repeated bouts of 1 min at 115% of $\dot{V}O_{2max}$ separated by 1 min rest, until ≥50 rpm could not be maintained) and overnight fasting. This is similar to the magnitude of change (22%) in the W' between rested and fatigued states measured in chapters 5, 6 and 7.

Combining chapters 6 and 7, 28 participants underwent a resting muscle biopsy and a muscle biopsy after 2 h of heavy-intensity exercise. This was immediately followed by a 3MT. Therefore, pooling these data provides an insight into the changes in muscle [glycogen] after 2 h of heavy-intensity exercise, and allows for correlations to be performed with the changes observed in EP and WEP. Muscle [glycogen] decreased by 66 ± 21% between rest and 2 h of heavy-intensity exercise (n=28; Figure 8.1).

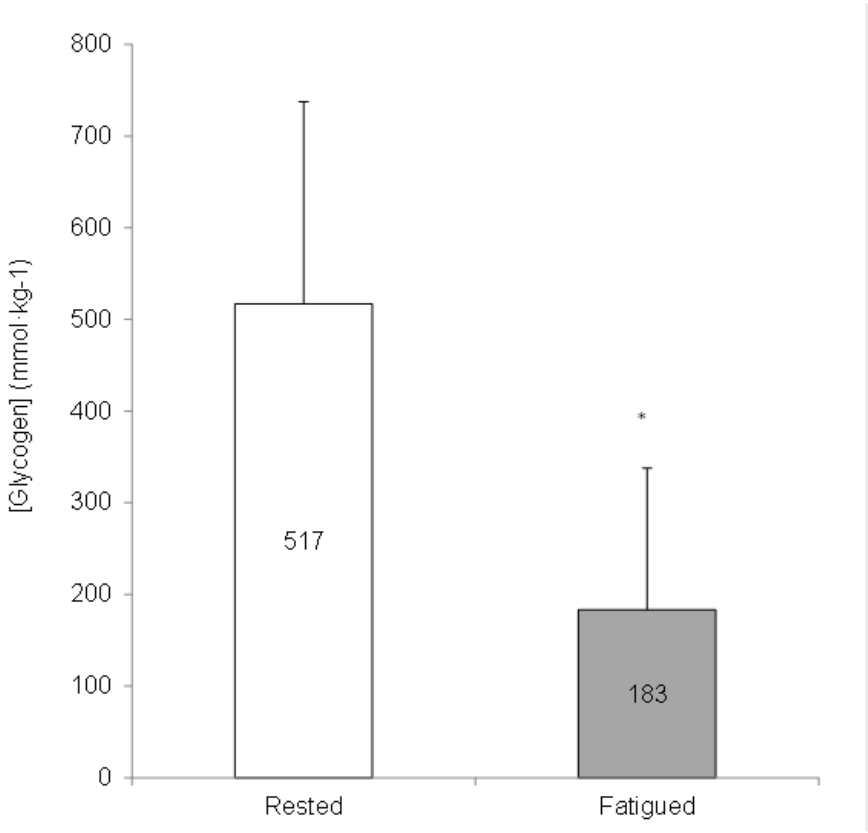


**Figure 8.1.** *Combined chapter 6 and 7 (n=28) muscle [glycogen] at rest and after 2 h of heavy-intensity exercise. * Different from rested condition (P < 0.001).*

The pooled data show that muscle [glycogen] at rest (517 ± 220 mmol·kg$^{-1}$ d.w.) correlated with control EP (272 ± 44 W; $r$ = 0.54; $P$ = 0.003; Figure 7.7A) and muscle [glycogen] post 2 h of heavy-intensity exercise (183 ± 155 mmol·kg$^{-1}$ d.w.) correlated with F-EP (245 ± 51 W; $r$ = 0.43; $P$ = 0.02; Figure 7.7B).

These results suggest that participants with a greater EP store greater concentrations of muscle glycogen (both at rest and post exercise). This suggests an association between aerobic fitness and greater muscle glycogen storage compared to untrained individuals (Costill et al. 1985). This is of interest and needs further investigation to understand the role stored muscle [glycogen] plays on EP. In addition, % changes in muscle [glycogen] (66 ± 21 %) over 2 h of heavy-intensity exercise correlated with the % change in WEP

(22 ± 15 %; *r* = 0.44; *P* = 0.02; Figure 8.2A) as well as absolute (kJ) change in WEP (3.9 ± 2.6 kJ; *r* = 0.43; *P* = 0.02; Figure 8.2B). Similarly, muscle [glycogen] post 2 h of heavy-intensity exercise (183 ± 155 mmol·kg$^{-1}$ d.w.) correlated with % change (*r* = 0.60; *P* = 0.001; Figure 8.2C) as well as absolute (kJ) change in WEP (*r* = 0.59; *P* = 0.001; Figure 8.2D).

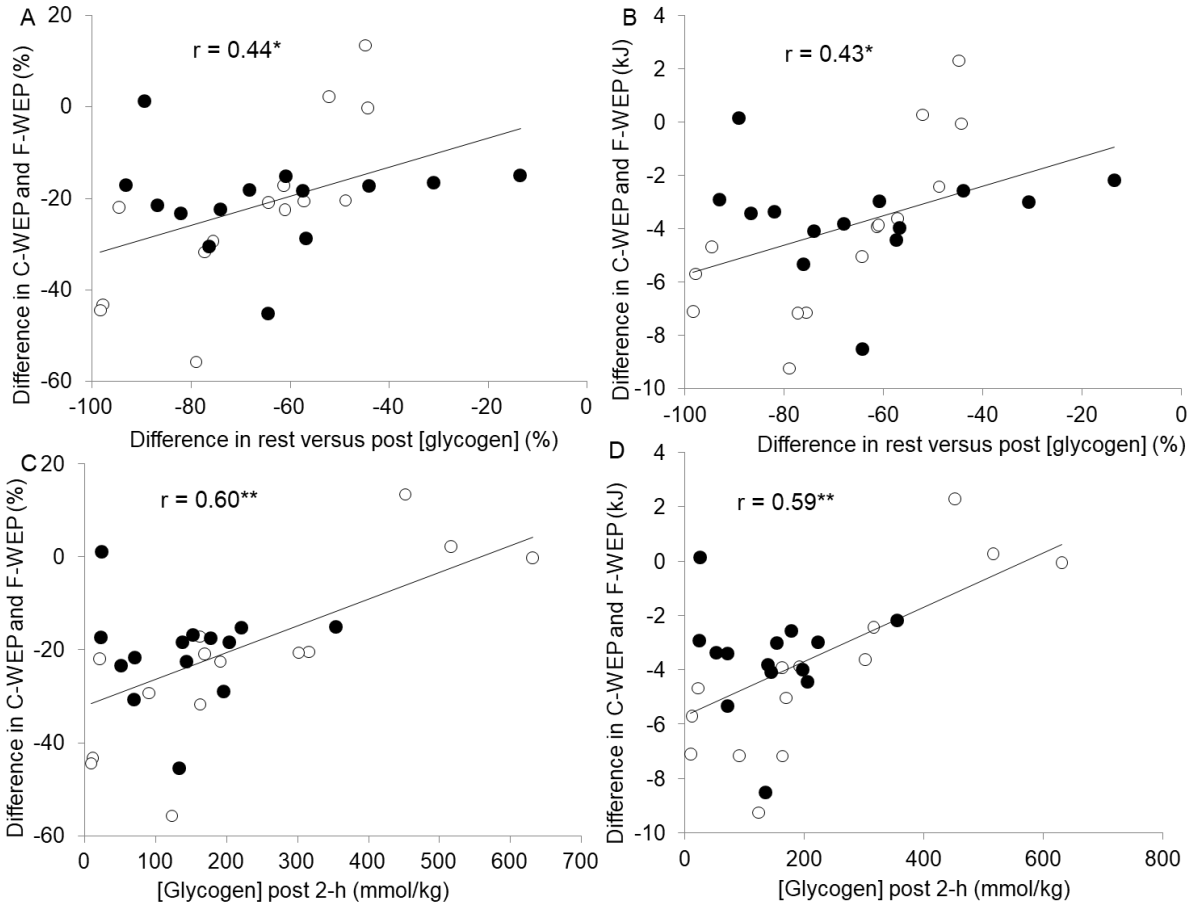


**Figure 8.2.** *Combined results from chapter 6 (white circles) and 7 (black circles) (n=28). Correlation between percentage change in muscle [glycogen] over 2 h of heavy-intensity and the percentage (panel A) and absolute (panel B) change in WEP over 2 h of heavy-intensity exercise. Correlation between muscle [glycogen] after 2 h of heavy-*

*intensity and the percentage (panel C) and absolute (panel D) change in WEP over 2 h of heavy-intensity exercise. * P* < 0.05.

These findings provide new insight into the relationship between muscle [glycogen] and WEP. The reduction in muscle [glycogen] over the course of prolonged fatiguing exercise is correlated with the reduction in WEP; the greater the reduction in [glycogen] the greater the reduction in WEP (chapter 6 and 7). Chapter 7 found that even when carbohydrate was consumed during prolonged exercise, the reduction in WEP and concurrent reduction in muscle [glycogen] remained. This suggests that the supplementation procedure used in Chapter 7 was not sufficient to spare muscle [glycogen]. However, there was greater carbohydrate availability and utilisation during exercise, evidenced by differences in blood [glucose] and exercising RER in the carbohydrate supplementation condition. However no correlations were evident between the changes in WEP and changes in muscle [glycogen] during prolonged fatiguing exercise, when carbohydrates were consumed. Further research is therefore needed to investigating the role muscle [glycogen] plays in WEP.

## 8.5 Experimental limitations

Chapter 4 investigated how a prolonged cognitive task affects exercise performance. It has been previously reported that the rating of perceived exertion (RPE) is affected during exercise that is preceded by a prolonged cognitive task (Marcora et al. 2009; Pageaux et al. 2013; Pageaux et al. 2014). RPE was not measured in chapter 4 to eliminate the focus disturbance RPE causes for the participants, but it is possible that there may have been an increase in RPE during the exercise bout that was preceded by

a cognitive task. While previous studies have reported that a 30 min cognitive task induced mental fatigue prior to exercise (Martin et al. 2016; Pageaux et al. 2014; Pageaux et al. 2015; Smith et al. 2016), in Chapter 4, there were no differences in response accuracy or response time over the duration of the cognitive task suggesting that the task might not been long enough to attain mental fatigue prior to the exercise task. Therefore future research is needed to explore the effect of longer or more arduous cognitive function tasks on exercise performance in athletes and untrained individuals.

Throughout the experimental chapters of this thesis, muscle biopsies were not taken prior to and post all experimental visits for ethical reasons. In Chapters 6 and 7, muscle [glycogen] was measured in participants at rest on one visit only (chapter 7 had biopsies during the carbohydrate visit as well as placebo), with the assumption that the participants had the same muscle [glycogen] prior to each visit. Furthermore, since participants completed exercise at the same intensity during all 2-h exercise bouts it was also assumed that a similar reduction in muscle [glycogen] would be present between visits. While participants were asked to record and repeat the same dietary and exercise behavior prior to each experimental visit, it is possible that small variations in muscle [glycogen] were present between these visits. It should also be noted that in chapters 6 and 7 there was a 60 s break between the 2 h heavy-intensity exercise bout and the 3MT for the attainment of muscle biopsies, with no break between the 2-h bout and the constant work rate bouts in chapter 6, such that there might have been some metabolic recovery (i.e. [PCr]) between the 2-h and the 3MT. However, firstly heavy-intensity exercise may not have an appreciable impact on [PCr] (Krustrup et al. 2004);

and secondly peak power output during the 3MT, which has been associated with PCr availability (Vanhatalo and Jones, 2009; Vanhatalo et al. 2016; Parker Simpson et al. 2012), did not change post 2 h of heavy-intensity exercise compared to control, suggesting that 2 h of heavy-intensity exercise with 60 s recovery did not significantly alter PCr availability.

### 8.6 Contributions to the area

This thesis has extended our understanding of the parameters of the power-duration relationship by investigating the effect that prolonged fatiguing exercise has on the CP and W′. This thesis provides novel insight into how estimates of exercise tolerance and performance might change during prolonged exercise. In particular, this thesis has demonstrated for the first time that both EP and WEP decrease after prolonged heavy-intensity exercise (Figure 8.3). Importantly, the extent of changes in these parameters were similar following 2 h exercise in chapters 5, 6 and 7 (reduction of 8-11% in EP and 20-22% in WEP).

Parker Simpson et al. (2012) found that 6 min of heavy-intensity exercise does not alter CP. Chapter 7 established that 40 min and 80 min of heavy-intensity exercise did not affect the EP, but that 2 h of heavy-intensity exercise did reduce EP. These results indicate that the decrease in EP might be detectable between 80 min and 2 h of heavy-intensity exercise. Parker Simpson et al. (2012) also found that 6 min of heavy-intensity immediately prior to a 3MT does not change W′. Similarly, chapter 7 found that 40 min of heavy-intensity exercise does not alter WEP whereas 80 min of prior heavy-intensity exercise reduced WEP. Additionally, chapters 5, 6 and 7 found that WEP is reduced

after 2 h of heavy-intensity exercise (Figure 8.3). These results suggest that a reduction in WEP might be detectable between 40 min and 80 min of heavy-intensity exercise. Additionally chapter 7 demonstrated that if carbohydrates are consumed during 2 h of heavy-intensity exercise the reduction seen in EP (but not WEP) after 2-h is eliminated.

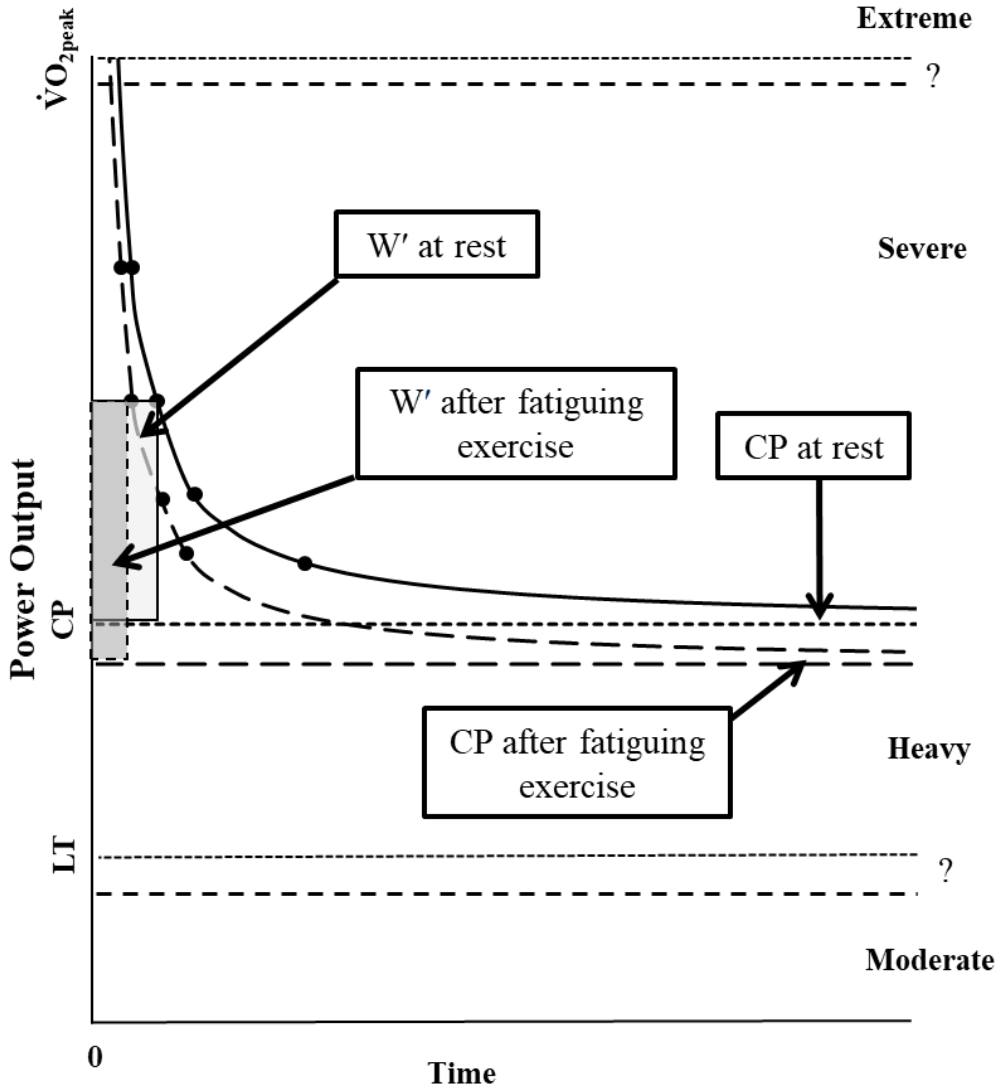


***Figure 8.3.*** *Schematic view of the power-duration relationship at rest and after 2 h of heavy-intensity exercise consuming only water. These are estimates from the combined results of chapters 5, 6 and 7. Note that CP and the rectangles (representing W') have been reduced after 2 h of heavy-intensity exercise compared to a rested state.*

Previous research has shown that the 3MT is both a reliable and valid test when performed with no prior exercise (Burnley et al. 2006; Vanhatalo et al. 2007). This thesis provides evidence for the first time that the 3MT provides reliable and valid estimates of EP and WEP when prolonged fatiguing exercise is performed prior to the administration of the test. Therefore the 3MT can be used in a fatigued setting to estimate a 'new' EP and WEP.

The combined results of the thesis provide insight into the behavior of both CP and W′ after 40 min, 80 min and 2 h of heavy-intensity exercise. This thesis gives us an understanding into the plasticity of the power-duration parameters during prolonged fatiguing exercise for the particular cohort of participants recruited.

## 8.7 Practical applications

The basic objective for an athlete competing in an endurance event is to cover a race distance in less time than their follow competitors or within a timeframe that elicits a personal record. Knowledge of CP and W′ prior to an event allows the athlete to optimise their race tactics. Moreover, knowledge of how these parameters change during an event allows for the formulation of appropriate countermeasures to maximise performance. Today we know that long endurance events are run or cycled below CS or CP respectively. Jones and Vanhatalo (2017) assessed elite marathon running speeds during a marathon and found that the athletes ran at 96% of CS. It is likely that these athletes consumed carbohydrates during their races to sustain such a high fraction of their CS. As the findings of this thesis suggest, CP may decrease by up to 11% during events lasting 2 h. If the athlete maintains the set race pace (~96% of CP), this implies

that at some point between 80 min and 2-h, CP will be surpassed and the athlete will be exercising within the severe domain, thus expediting fatigue (Figure 8.4).

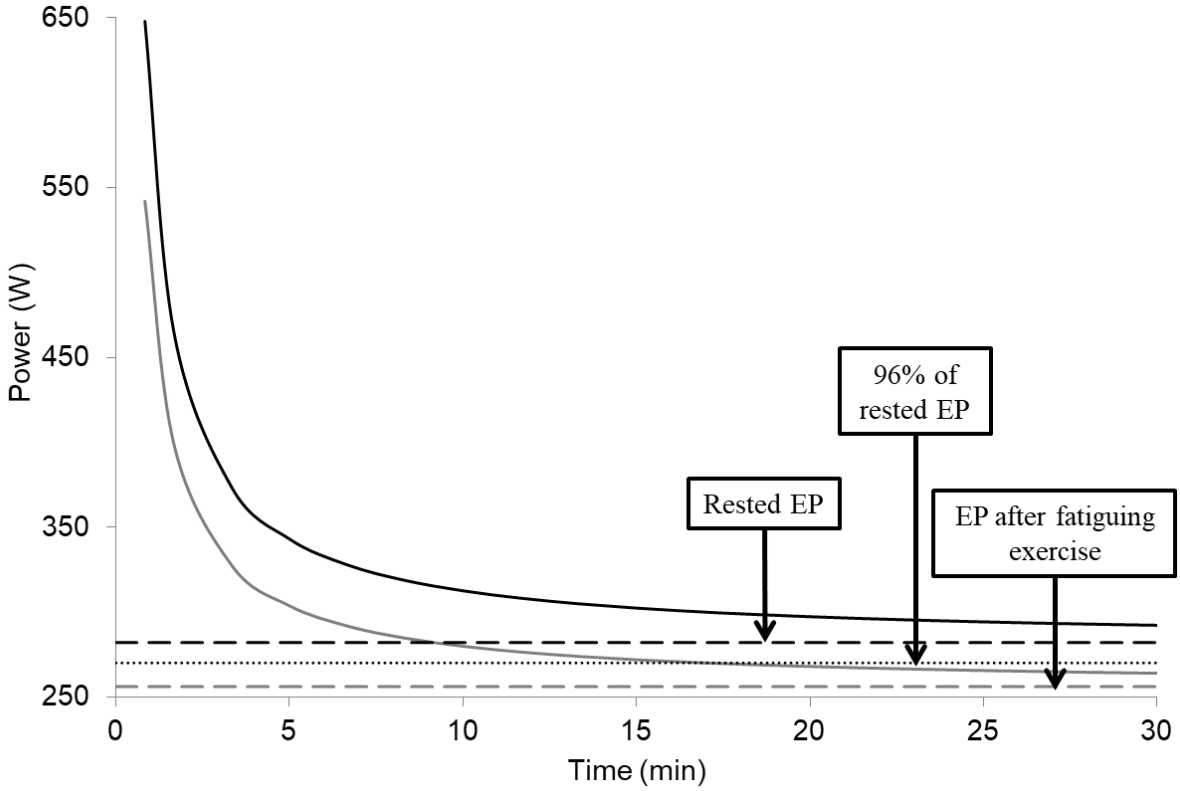


***Figure 8.4.*** *Hyperbolic curves estimated of combined EP and WEP from chapter 6, 7 and 8 (n=42). Black solid line represents the hyperbolic curve estimated from a rested state and grey line represents the hyperbolic curve estimated following 2 h of heavy-intensity exercise (~9% decrease in EP and ~22% decrease in WEP). Dashed black line represents rested EP, dashed grey line represents EP after 2 h of heavy-intensity exercise and small dotted line represents 96% of rested EP.*

However, the results of this thesis indicate an appropriate carbohydrate supplementation strategy during events lasting more than 2-h might allow the athlete to maintain the set race pace until completion by eliminating the transition into the severe-

intensity domain. With this in mind, rested CP estimates can be used to predict performance of races lasting up to 80 min or 2-h provided that 60 $g \cdot h^{-1}$ of carbohydrates is consumed. While CP might be maintained during events in which carbohydrate is consumed, the findings of this thesis suggest that a decrease in W′ may be experienced during long duration events which cannot be abolished by carbohydrate supplementation. This has implications for planning a sprint finish in that the remaining fraction of the W′ estimated at rest will allow for an effort above CP that is proportionate to this fraction (Figure 8.4).

## 8.8 Future directions

In chapter 7, 60 $g \cdot h^{-1}$ of carbohydrate supplementation eliminated the reduction seen in EP but not in WEP. This provides a basis for further investigation to investigate optimal carbohydrate supplementation procedures to combat changes in these parameters. In a glycogen depleted state, chapters 5, 6 and 7 of this thesis showed a similar reduction in WEP to that observed by Miura et al. (2000). During the carbohydrate consumption trial of chapter 7, muscle [glycogen] was significantly reduced despite the higher blood [glucose] compared to the un-supplemented trials. A greater level of glucose oxidation was evident during the carbohydrate consumption trial in chapter 7, which may explain the reduction seen in muscle [glycogen]. It is possible that the higher plasma glucose observed in the carbohydrate compared to the placebo condition (chapter 7) lessened central fatigue development (Coggan and Coyle, 1991; Nybo, 2003), and in turn increased muscle activation in the subsequent 3MT eliminating the decrease seen in CP when no carbohydrates where ingested. Additionally, it has been shown that

carbohydrate mouth rinse attenuates the decrease in exercise performance (see chapter 2.4.2.5) and this could also have a positive effect on CP after prolonged exercise. W′ has been proposed to reflect the metabolic, contractile and fatigue related characteristics of type II muscle fibers (Vanhatalo et al. 2011b; Vanhatalo et al. 2016). Therefore the decrease in WEP following prolonged exercise, irrespective of supplementation, may reflect the specific effect, such as the decrease in muscle glycogen, of prolonged exercise on type II muscle fibers. While it seems possible that EP may be modulated by central fatigue and the changes in WEP could be related to muscle glycogen depletion, future investigations should explore which muscle metabolic, cardiovascular and/or neuromuscular factors underlie the decline in CP and W′ after prolonged fatiguing exercise.

Future studies should investigate the behavior of CP and W′ in different groups of athletes in order to best predict how particular athletic populations might perform during long-duration events. Additionally, future studies might investigate the effect of prolonged heavy-intensity exercise on other established exercise thresholds used in performance modelling including the CS, D′, LT and the power output eliciting $\dot{V}O_{2max}$ after prolonged fatiguing exercise (Figure 8.3). The current thesis shows that the heavy-severe boundary shifts after prolonged exercise. However, it is not clear how the exercise intensity domains should be defined in a fatigued state. It is possible that the thresholds separating the domain and the physiological responses to constant work rate exercise might be assessed differently in a fatigued versus a rested state.

In chapters 5, 6 and 7, participants exercised at 25%Δ1 before estimating the parameters of the power-duration relationship. It is possible that a higher or a lower

fixed intensity within the heavy-intensity domain would result in greater or a smaller change in EP and WEP. For instance elite marathon runners have been shown to run at 96% of CS (Jones and Vanhatalo 2017), and therefore a higher fixed intensity during the constant work rate bout could result in a greater change in D′ (and possibly CS if no supplements are consumed) and would be of interest to investigate. Additionally it may be useful to assess the changes in CP and W′ over a specific time trial or distance in events such as the tour de France.

## 8.9 Conclusion

The findings of this thesis have provided novel insight into the role of both psychobiological and physiological factors in limiting exercise performance. In summary, the findings of this thesis demonstrate that, firstly, TT performance of trained athletes or untrained individuals is not affected by prolonged cognitive tasks; and, secondly, that both EP and WEP are reduced after prolonged fatiguing exercise. The understanding of the changes in EP and WEP after prolonged fatiguing exercise provides a basis for improved accuracy of performance predictions during endurance events and sets a framework for developing countermeasures to offset the ergolytic effects of these changes.

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